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Foliar Diagnosis of Mineral Status of Hevea in Relation to Bark Analysis

E. W. BOLLE-JONES

Leaf sampling and diagnosis procedures are discussed and compared with results obtained by the analysis of bark for the assessment of the response of mature rubber trees to fertilisers. Significant correlationships were established between the bark and lamina concentrations of many of the nutrient elements investigated. Because of the difficulties of leaf sampling it is suggested that bark composition may become a means of evaluating the mineral nutrient status of mature trees. A diurnal variation in the concentration of magnesium is shown to occur in the laminae.

THE CHEMICAL COMPOSITION OF LEAVES, which readily show symptoms of nutritional disorders, may be employed as a method of diagnosis of mineral status; comparison is made with values obtained for leaves which either exhibit known deficiency symptoms or are healthy. To apply this approach towards the estimation of the nutrient status of a crop grown in the field requires a standardisation of sampling positions and samples which are representative of the area under examination. For young rubber plants, up to the unbranched stage, sampling is relatively easy as the leaves are found in well defined storeys, the uppermost three of which are separately sampled and combined with corresponding storeys from other plants which also contribute towards the 'bulk' sample used for analysis. This method of sampling, based on storey position, automatically takes into account the relative maturity of the leaves for plants similar in regard to age and growing conditions. Nevertheless, to make a diagnosis based on the analyses of such samples requires some measure of experience as storey or whorl position markedly influences the concentration of the foliar constituents, the effect of storey position on chlorophyll and certain minerals may vary according to the time of year, and for some mineral nutrients significant storey × treatment interactions are obtained (BOLLE-JONES 1954b, BOLLE-JONES & RATNASINGAM 1954).

Little is stated in the literature on the sampling of hevea leaves but a statement (CHAPMAN 1941) which indicated that a diurnal variation of foliar constituents occurred and that it was necessary to carry out leaf sampling at fixed times, between sunrise and 10.30 hours. This statement was not supported by experimental evidence and it was thought necessary to confirm its validity. The first section of this paper examines the variation, during the course of the day, of the mineral nutrients within the leaves of young hevea plants. It was found, for nitrogen, phosphorus, potassium, calcium and manganese, that there was no evidence of any significant variability associated with the time of day. However, the foliar magnesium concentration showed a significant diurnal variation; the values fell markedly after midday but increased towards evening. Diurnal changes also occurred in the iron concentration but they were not generally consistent from day to day. It seemed that the best time to sample leaves was before 10.00 hours. It was assumed that a similar variation occurs in mature trees.

After this stage in the investigations had been reached it was concluded that a reasonably satisfactory method of diagnosis of mineral status was available for young hevea trees up to the one year old stage. Thus it was preferable to take leaf samples in the

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forenoon and they should be taken according to storey position. The number of leaves which comprised any one sample was not less than forty and they were derived from a minimum of five trees which number could be increased according to the area being assessed and the number of replicates required for analyses. Samples were taken from healthy and affected areas and the diagnosis took into consideration any visual symptom apparent in the unhealthy leaves. A knowledge of these symptoms (BOLLE-JONES 1956) and the changes in foliar chemical composition which accompany their appearance, has proven a valuable aid in the assessment of mineral nutrient status of hevea plants.

However, the application of foliar diagnosis techniques to tall mature trees posed many more difficult problems, such factors as: the height of the sampling position above ground, the part of the crown to be sampled in regard to sun and shade positions, the ages of individual leaves and trees, and lastly the inaccessibility of the selected leaves. CHAPMAN (1941) sampled from spur shoots of limited growth on the tree; the largest leaf was taken from each of two well developed storeys 'high up in the light and on opposite sides of the tree'. Probably many trees were sampled in such fashion and all leaves bulked before analysis. BEAUFILS (1955) adopted a similar system and sampled the three most mature leaves of any one shoot of limited growth; these 'boughs are located inside the crown and grow generally on main or primary limbs.' About 40 leaves, taken from a minimum of 15 trees comprised one sample and all sampling was carried out between 07.00 and 10.30 hours. Apart from the manifest difficulty in choosing such few representative leaves out of the many hundreds present in any area to be examined, there is the hazard inherent in their collection* and the long time required to collect even one sample. For such reasons, this method of assaying the nutrient status of mature trees has never been favoured by the author but compulsorily adopted in the absence of any other well investigated alternative. Two possibilities presented themselves: the use of the bark composition as a method of diagnosis, and to relate mineral nutrient status to the composition of the latex. The former has been investigated and the results follow. Examination of the analyses of leaf and bark samples from field manurial trials showed that, in many respects, analysis of bark gave as good an indication of plant nutrient status as did that of leaves. The ease and rapidity of bark sampling and its amenity to standardisation may lead to its adaptation for the assessment of mineral status of mature trees.

DIURNAL VARIATION IN CONCENTRATION OF NUTRIENT ELEMENTS IN LAMINAE

Method of Investigation

It was planned to analyse leaves sampled at 2 hourly intervals, between 06.00 hours and 22.00 hours, from young nursery grown plants. It was anticipated that the amplitude of any diurnal variation would be small so that any attempt at its detection would require well standardised conditions. As all the samples could not be killed and oven dried immediately after being taken, the technique must allow for the leaves to be stored for some period of time, without deterioration, before being finally oven dried. If, for instance, the leaves were to be removed from the trees at 16.00 hours and left until the following morning before being killed, some loss in dry weight might occur, due to respiration, and the mineral concentration values obtained might appear higher than they actually were. One technique, which appeared promising and convenient to operate, was to kill the leaf tissue by dipping it in liquid air and then to store the killed tissue in a refrigerator until such time as it could be oven dried. To test the validity of such a procedure the following trial was carried out.

* CHAPMAN (1941) stated, in regard to leaf sample collection: 'This work was carried out by gangs of coolies who were directed from the ground when cutting spurs'.

Eight plants of *Hevea brasiliensis* clone RRIM 604, not more than a year old and as much alike as possible, were selected; they were grouped into 4 pairs. The terminal bud was quiescent and leaves of the top storey were mature and fully expanded. All the leaves of the top storey were removed, for analysis, from each pair, which was regarded as one unit or replicate; the first replicate was sampled at 08.15 hours and the subsequent ones at 08.45 hours, 09.15 hours and 09.45 hours. Immediately after all the top storey leaves were removed the midrib and petiole were cut out and discarded and the residual lamina, of each leaf, was quartered. All the quarter sections of lamina for one replicate were mixed and then placed at random in one of 4 beakers. The first beaker — treatment W — was immediately placed in the oven and its contents dried at 80°C in a current of air. The second beaker — treatment X — contained liquid air and the lamina sections were frozen almost immediately on being placed in the beaker, the beaker was then transferred to an oven and the lamina material dried as for treatment W. The third beaker — treatment Y — also contained liquid air; the lamina sections were frozen and the beaker subsequently placed in a refrigerator for 6 hours before its contents were oven dried. The fourth beaker — treatment Z — also contained liquid air in which the lamina sections were frozen; the beaker was kept in a refrigerator for a further 24 hours before its contents were oven dried.

TABLE 1. EFFECT OF FREEZING AND SUBSEQUENT DRYING ON CONCENTRATION OF NUTRIENT ELEMENTS IN LAMINAE OF HEVEA BRASILIENSIS

Clone RRIM 604 top storey laminae. Results expressed in terms of dry matter of laminae.
W immediately oven dried
X frozen, then dried
Y frozen, stored in refrigerator for 6 hours, then dried
Z frozen, stored in refrigerator for 24 hours, then dried

Treatment	N%	P%	Mg%	K%	Ca%	Fe p.p.m.	Mn p.p.m.
W	3.03	0.24	0.22	1.11	0.64	76	89
X	2.95	0.24	0.23	1.15	0.65	79	88
Y	2.94	0.24	0.23	1.16	0.65	75	90
Z	2.94	0.22	0.24	1.15	0.67	73	90
Min. 5% sig. diff.	0.08	0.01	0.01	0.05	0.02	6	7

When dried, each sample was ground and analysed according to methods previously described (BOLLE-JONES 1954a); the results are summarised in Table 1. It appeared that there was no significant difference between the effects of treatments X and Y on the concentration of all the nutrient elements examined. For nitrogen, magnesium, potassium, manganese and iron this statement could be extended to include treatment Z. However, the phosphorus and calcium concentrations of samples subjected to treatment Z differed significantly from those of treatments X and Y. Treatment W gave different results from X, Y and Z for nitrogen and potassium.

In view of these results, it was decided to freeze all laminae sampled during the investigation of diurnal variation and to oven dry as soon as convenient after freezing. This method would ensure that the results were not influenced by technique as far as

TABLE 2. DIURNAL VARIATION IN CONCENTRATION OF NUTRIENT ELEMENTS IN LAMINAE

Top storey laminae sampled at intervals of 2 hours on three separate days; Tjir 1 plants sampled on 22 October and Clone RRIM 604 on 18 and 19 December 1952. Triplicate samples taken at each sampling time. Results expressed in terms of dry matter of laminae.

ANALYSIS OF VARIANCE: MEAN SQUARES

Source	d.f.	Nitrogen	Phosphorus	Magnesium	Potassium	Calcium	Iron	Manganese
Days	2	0.8020	0.00108	0.00065	0.0525	0.6043*	13220***	7257.0
Times (of sampling)	8	0.0106	0.00025	0.00043***	0.0019	0.0051	859	76.6
Days x times	16	0.0069	0.00031	0.00008	0.0030	0.0058	734***	43.1
Replicates within days	6	0.1654***	0.01445***	0.00229***	0.3456***	0.0785***	229**	1473.6***
(Replicates x times) within days	48	0.0073	0.00042	0.00006	0.0029	0.0045	66	37.3
Pooled error	64	0.0072	0.00039	0.00006	0.0029	0.0048	—	38.8
Coefficient of variation (%)		2.6	9.1	4.2	4.5	8.3	8.6	6.4

* 5% sig. **1% sig. *** 0.1% sig.

MEAN VALUES

Time	N%	P%	Mg%	K%	Ca%	Fe p.p.m.	Mn p.p.m.
6.00 hours	3.28	0.22	0.20	1.23	0.83	114	102
8.00 hours	3.33	0.23	0.19	1.21	0.81	103	99
10.00 hours	3.29	0.22	0.19	1.20	0.86	101	101
12.00 hours	3.26	0.22	0.19	1.21	0.83	93	96
14.00 hours	3.27	0.22	0.18	1.20	0.83	85	94
16.00 hours	3.23	0.22	0.18	1.21	0.80	86	95
18.00 hours	3.33	0.23	0.18	1.22	0.88	85	100
20.00 hours	3.31	0.23	0.19	1.19	0.85	91	98
22.00 hours	3.32	0.23	0.19	1.23	0.83	93	94
Min. 5% sig. diff.	0.08	0.02	0.01	0.05	0.07	27	6

nitrogen, magnesium, potassium, manganese and iron were concerned but that for phosphorus and calcium the results should be interpreted with caution. It subsequently ensued that during the examination of diurnal variation effects none of the lamina samples was left more than 14 hours before being oven dried. This was a period of 8 hours more than allowed in treatment Y but 10 hours less than treatment Z.

The examination of diurnal variation effects was carried out in the following fashion. Leaf samples were taken at 2 hourly intervals on three separate days: 22 October 1952, 18 December 1952 and 19 December 1952. Tjirandji 1 seedling plants were sampled on 22 October and RRIM 604 plants on the remaining two days; all plants were grown in an uniform nursery area and were about one year old. Sampling was carried out in triplicate; each sample was derived from two trees which appeared to be almost of identical appearance. Each tree possessed fully expanded uppermost storey leaves and the terminal bud was quiescent. From each member of a pair of trees, one leaf (that is, three leaflets) was removed at each sampling time from the topmost storey and combined for analysis — the sequence of leaf removal being consistently basipetal for one member of the pair and acropetal for the other. This procedure was executed at the following hours: 06.00, 08.00, 10.00, 12.00, 14.00, 16.00, 18.00, 20.00 and 22.00, so that 9 leaves were removed from the uppermost storey of each selected plant.

After sampling the petioles and midribs were discarded, the laminae frozen in liquid air and stored in the refrigerator until taken later for oven drying.

Results

Significant variability, according to time of day, did not occur in the concentrations of nitrogen, phosphorus, potassium, calcium and manganese found in the lamina (Table 2). However, the magnesium concentration showed a significant diurnal variation; the values fell after midday and increased again towards evening. The absence of a significant days × times interaction demonstrated that the diurnal change in magnesium concentration was similar on all three sampling days. Diurnal changes also occurred in the iron concentration but the variation was not consistent for each of the three days. On the first day there was a general decrease in the iron concentration as the day progressed, with the most marked decrease occurring between noon and 14.00 hours. On the second day an initial decrease occurred but thereafter the changes were less pronounced; the lower values occurred at noon and 14.00 hours. On the third day the diurnal changes were not significant. These diurnal changes are shown in Table 3.

TABLE 3. DIURNAL VARIATION IN CONCENTRATION OF IRON IN THE LAMINAE

For details see Table 2. Day x times (hours). Iron concentration, means (p.p.m.)

Clone and day	6.00	8.00	10.00	12.00	14.00	16.00	18.00	20.00	22.00	Day means
Tjir 1 on 22 October	160	151	136	135	104	97	97	97	102	120
RRIM 604 on 18 December	101	82	81	66	74	84	86	91	91	84
RRIM 604 on 19 December	82	75	86	77	78	76	72	84	86	80
Min. 5% sig. diff.					13					17

Other observations, made during the present study, indicated (Table 2) that the concentration of calcium in the lamina may vary according to the day of sampling. The same could not be said of iron concentration, which also showed a significant day

effect, as the data for the first day, which gave substance to this significance, was derived from different clonal material to that of succeeding two days. It has been shown elsewhere (Bolle-Jones & Ratnasingam 1954) that different clones are characterised by different iron concentrations in the laminae.

Conclusion

If consideration is given to the significant diurnal variation of magnesium concentration then the best practical time of day for leaf sampling is before 10.00 hours. Maximum concentration values are obtained up to this time limit and the drop of values soon after noon is avoided. Similarly for iron an early sampling will tend to yield higher values and avoid the afternoon drop. Taking all the nutrient elements, as estimated here, into consideration it can be seen (Table 2) that a sampling period from 06.00 to 10.00 hours is usually satisfactory and can be recommended for general use. Whenever possible this recommendation was followed.

COMPARISON OF BARK ANALYSIS WITH LEAF ANALYSIS AS A DIAGNOSTIC PROCEDURE

Methods of Investigation

When dealing with tree crops, sampling of leaves for diagnostic purposes may often present a difficult problem because of variations in chemical composition according to age of leaf, diurnal and seasonal fluctuations and, above all, the practical difficulty of collecting representative samples from tall mature trees. The possibility of assessing nutrient status of trees by analysing bark samples is considered below. The requirement was the practical one of determining responses to fertiliser addition by trees grown in manurial trials; it is hoped that the initial findings reported here may be extended to become a means of diagnosis of mineral status.

It was necessary, as a first step, to take samples of bark at varying heights to determine whether position of sampling influenced composition. Two 14 years old trees, grown from basal cuttings made from unselected seedlings, were used for this test and bark was removed at heights of 5, 10, 15, 20 and 25 feet above ground level. The bark was removed in slivers down to the wood. A similar method to that employed for later sampling trials was used and is described below. The composition of the bark samples as affected by height is given in Table 4.

Table 4. Variation of Bark Composition According to Height of Sampling Point

Concentration values given in terms of oven dried bark

Height above ground	Ash%	N%	P%	K%	Ca%	Mg%	Mn p.p.m.
5 ft	11.4	0.41	0.042	0.85	3.77	0.12	74
10 ft	10.4	0.42	0.044	0.78	3.48	0.16	75
15 ft	11.2	0.44	0.046	0.93	3.68	0.20	80
20 ft	10.4	0.46	0.046	0.93	3.32	0.18	69
25 ft	10.1	0.50	0.053	0.84	3.25	0.19	69
Min. 5% sig. diff.	1.4	0.10	0.016	0.21	0.57	0.05	19

Ash, nitrogen, phosphorus, potassium, calcium and manganese concentration did not vary according to height. However, the magnesium concentration was significantly influenced by height of sampling but there was little indication of a simple relationship between the magnesium concentration and height. It was concluded that varying height was a relatively unimportant source of variation in the composition of the bark and it was decided to fix on an arbitrary and convenient height for all future investigations. The analysis of variance, which is not presented, also showed that replicate samples taken at different heights on the same tree did not provide more information than was obtained from one sample taken per tree; duplicate determinations on a given sample were also superfluous.

Three manurial trials, which formed part of an extensive programme of field experiments (OWEN, WESTGARTH & IYER 1957), and which had shown yield and girth responses to fertiliser application, were selected for these investigations. The trees were more than 15 years old. A maximum of 100 (Seaport Estate trial) and a minimum of 20 (Field 31 trial) trees, at a density of about 100 trees per acre, occurred per plot. Of this number, 5 trees only, which were centrally located and chosen to avoid poaching effects, were selected for leaf and bark sampling purposes; this number may be considered small but for a well replicated experiment, of the type examined, it proved to be adequate.

Sections of the bark, four inches by one inch, taken down to the wood, were removed at a height of 90 inches (Field 31 and Wardieburn Estate trials) or 100 inches (Seaport Estate trial) above the union. This sliver of bark (the 4 inches being in the vertical plane) was taken from the side of the trunk away from the tapping cut. On excision, the section was sprayed with 15% v/v formic acid solution, to coagulate the rubber, and then placed in clean cotton bags. The samples were oven dried, powdered and then analysed for the nutrient elements, according to methods previously described (BOLLE-JONES 1954a).

From each tree selected for sampling, approximately 20 leaves were removed, they were neither very small nor shade leaves and they were borne on the lower region of the spur or lateral branches. These branches were borne as high up in the crown as could be reached by an Indian boy climber, who was equipped with a very long handled knife. As far as possible, 10 leaves were picked on one side of the crown and 10 leaves on the opposite side; a total of 100 leaves per plot was, therefore, collected. These leaves were returned to the laboratory as soon as possible, the midribs excised and discarded, the laminae oven dried and then ground to a powder before mineral analysis.

Each manurial trial contained at least six fertiliser treatments, sometimes as many as twelve, and a minimum of two replicates was sampled. This meant, in practice, that a leaf and bark sampling of any one trial took four days. On the first day leaf samples were taken in the early morning; bark was sampled from the same trees on the following day. On the third morning the remaining replicates were leaf sampled to be followed by the corresponding bark sampling on the fourth day.

To collect one leaf sample (that is, leaves from 5 trees) required the attention of one field assistant and two boy climbers and took about one hour. Bark sampling (from the same number of trees) was much more rapid and was easily completed by one field assistant within half an hour.

Results

Field 31 Trial (Rubber Research Institute Experiment Station) — This experiment consisted of 6 manurial treatments in a latin square design; control, n, nk, np, pk and npk; the trees were budded in 1937 and were first tapped in 1947. Prior to the present

Table 5. Field 31 Trial

Concentration of nutrient elements in dried laminae and girth of trees at sixty inches measured in December 1954

TREATMENT MEANS

Treatment	Ash%	N%	P%	Mg%	K%	Ca%	Fe p.p.m.	Mn p.p.m.	Girth cm
(1) Control	4.14	3.19	0.15	0.23	1.18	0.49	96	114	82.2
n	4.04	3.02	0.15	0.19	0.97	0.58	107	93	76.5
np	3.96	3.22	0.18	0.22	1.19	0.39	91	77	89.2
nk	3.96	3.27	0.15	0.18	1.20	0.38	96	56	81.4
pk	3.94	3.15	0.18	0.22	1.08	0.49	100	76	86.9
npk	4.07	3.11	0.18	0.20	1.15	0.46	99	104	87.0
Min. 5% sig. diff.	0.53	0.27	0.03	0.05	0.30	0.19	16	75	7.5

MEAN EFFECTS

Effect	Ash	Nitrogen	Phosphorus	Magnesium	Potassium	Calcium	Iron	Manganese	Girth
N	–0.0338	–0.0160	–0.00125	–0.0275	–0.00125	–0.0388	0.125	–12.375	–1.038
P	–0.0431	–0.0119	0.03125**	0.0200	0.01625	–0.0269	–3.812	3.750	7.721**
K	–0.0481	0.0406	0.00125	–0.0200	0.02375	–0.0319	1.438	–16.875	–0.119
95% conf. limit	0.3263	0.1648	0.01938	0.0313	0.18657	0.1184	9.482	46.166	4.560
99% conf. limit	—	—	0.02680	—	—	—	—	—	6.306

Table 6. Field 31 Trial

Concentration of nutrient elements in dried bark and yield of rubber in grams per tree per tapping during October 1954 to February 1955

TREATMENT MEANS

Treatment	Ash%	N%	P%	Mg%	K%	Ca%	Fe p.p.m.	Mn p.p.m.	Rubber gm
(1) Control	6.05	0.51	0.030	0.12	0.88	1.66	46	174	106
n	6.29	0.54	0.030	0.12	0.64	1.90	53	159	84
np	5.73	0.58	0.042	0.12	0.97	1.48	54	147	150
nk	5.00	0.53	0.029	0.10	0.82	1.32	44	124	110
pk	6.12	0.54	0.046	0.10	0.88	1.72	48	134	121
npk	6.88	0.53	0.044	0.13	0.88	1.93	53	195	117
Min. 5% sig. diff.	2.17	0.07	0.008	0.04	0.27	0.92	17	93	50

MEAN EFFECTS

Effect	Ash	Nitrogen	Phosphorus	Magnesium	Potassium	Calcium	Iron	Manganese	Rubber
N	–0.1088	0.0206	–0.00212	0.0094	–0.04875	–0.0350	3.750	2.562	1.819
P	0.5288	0.0294	0.01375***	0.0088	0.13188	0.1081	5.438	10.688	31.519*
K	0.2012	–0.0206	0.00150	–0.0112	–0.01562	–0.0619	–4.812	–12.562	8.031
95% conf. limit	1.3302	0.0438	0.00520	0.0255	0.16351	0.5638	10.648	57.201	30.375
99.9% conf. limit	—	—	0.00995		—	—	—	—	—

sampling the respective fertilisers had been applied to each plot in 1947, 1949, 1951 and 1953. Tjirandji 1 trees grown in replicates 1, 2, 5 and 6, were sampled for the purposes of this investigation. Sampling was carried out in early December 1954. The yield of rubber per tree per tapping recorded for the period October 1954 to February 1955 showed a significant beneficial effect due to the application of fertilisers containing phosphorus. A similar significant improvement of girth measurement due to phosphate addition was detected in the girth measured in December 1954.

Summaries of the effects of fertiliser treatments on girth measurement, yield of rubber and mineral compositions of laminae and bark are given in Tables 5 and 6. The chief feature was that the mean effect of the phosphate fertiliser on the concentration of phosphorus in the tissues was equally reflected in bark as well as in lamina composition. It was also evident that whenever phosphate fertiliser had been included in any treatment, the concentration of phosphorus in the bark tissue showed relatively greater increases than those found in the laminae.

Wardieburn Estate Trial — This trial was laid out as $2 \times 2 \times 2$ factorial experiment in which two levels (with and without) of nitrogen–, phosphorus–, and potassium–, containing fertilisers were applied in all combinations, and with five replications. The Tjirandji 1 trees grown in this experiment were budded in 1939 and first tapped in 1946; each treatment received its respective fertiliser application in 1948, 1950 and 1952. Leaf and bark samplings were carried out on trees grown in replicates B and C of this trial towards the end of August 1955. Girth measurements taken at a height of 72 inches above the union and recorded in July 1955 showed a significant positive effect of phosphate application. The average yield of rubber per tree per tapping, recorded during the period June to October 1955, was significantly depressed whenever potassium had been applied, except for the npk treated trees; a very definite negative mean effect due to potassium was recorded. However, yield records recorded during the period September 1954 to August 1955 inclusive showed that phosphate addition had a significant beneficial influence on yield although this effect did not achieve significance for the average based on the more limited June to October 1955 period. Summaries of most of these effects and the mineral compositions of laminae and bark are given in Tables 7 and 8.

The mineral analyses showed that phosphate, not only favourably influenced girth and yield production, but also produced significant mean effects on the concentrations of phosphorus and calcium in the laminae and bark. A significant mean effect of phosphate application was also noted on iron and manganese concentrations in the laminae but not for bark, and vice versa in regard to ash. Consequently the bark and laminae did not mirror the mean effects of phosphate addition in the same way or to the same extent.

Where nitrogen fertiliser only had been applied a negative effect on the manganese concentration of both bark and laminae was produced but it was only the laminae which showed a significant positive mean effect on nitrogen concentration when all treatments were taken into consideration. Thus the laminae registered more sensitively the addition of nitrogen fertilisers than did the bark.

Neither bark nor lamina composition indicated any significant effect attributable to the addition of fertilisers containing potassium.

Seaport Estate Trial — This was a factorial experiment in which three levels of potassium were applied, with two replications. Trees of clone PB 25, which were budded in 1938, were grown in this trial; each fertiliser treatment was applied in 1951, 1953 and 1955, and the trees had been tapped since 1947. Leaf and bark sampling were carried out on trees of both replicates (that is a total of 24 plots) in early

Table 7. Wardieburn Estate Trial

Concentration of nutrient elements in dried laminae and girth of trees at seventytwo inches measured in July 1955

TREATMENT MEANS

Treatment	Ash%	N%	P%	Mg%	K%	Ca%	Fe p.p.m.	Mn p.p.m.	Girth cm
(1) Control	4.31	3.15	0.18	0.18	1.22	0.52	78	118	67.0
n	3.89	3.32	0.16	0.12	1.29	0.38	74	56	63.8
p	4.74	3.13	0.20	0.13	1.44	0.70	76	164	71.0
np	4.15	3.35	0.25	0.12	1.04	0.72	88	144	69.3
k	4.37	3.19	0.17	0.13	1.38	0.50	78	92	63.6
nk	4.16	3.38	0.18	0.12	1.40	0.40	76	98	64.0
pk	4.64	3.09	0.18	0.12	1.44	0.64	71	130	67.5
npk	4.82	3.22	0.22	0.19	1.45	0.67	96	132	69.7
Min. 5% sig. diff.	1.05	0.29	0.04	0.12	0.39	0.08	10	41	8.4

MEAN EFFECTS

Effect	Ash	Nitrogen	Phosphorus	Magnesium	Potassium	Calcium	Iron	Manganese	Girth
N	–0.2600	0.1775*	0.0192	0.0000	–0.0725	–0.0475*	7.625**	–18.50	–0.608
P	0.4075	–0.0600	0.0435**	0.0025	0.0225	0.2325***	5.875*	51.75***	4.768*
K	0.2250	–0.0150	–0.0075	0.0050	0.1700	–0.0300	0.875	– 7.50	–1.565
95% conf. limit	0.5229	0.1448	0.0214	0.0589	0.1950	0.0399	5.026	20.27	4.193
99% conf. limit	—	—	0.0316	—	—	0.0590	7.435	30.00	—
99.9% conf. limit	—	—	—	—	—	0.0912	—	46.33	—

Table 8. Wardieburn Estate Trial

Concentration of nutrient elements in dried bark and yield of rubber in tahils per tree per tapping during September 1954 to August 1955

TREATMENT MEANS

Treatment	Ash%	N%	P%	Mg%	K%	Ca%	Fe p.p.m.	Mn p.p.m.	Rubber (tahils)
(1) Control	5.84	0.50	0.041	0.058	0.71	1.70	50	190	0.82
n	3.89	0.56	0.034	0.034	0.69	0.98	46	146	0.75
p	6.54	0.50	0.058	0.034	0.88	1.90	54	188	0.76
np	6.56	0.54	0.058	0.016	0.59	2.14	53	184	0.84
k	4.86	0.52	0.034	0.037	0.90	1.16	52	188	0.71
nk	4.60	0.56	0.028	0.026	0.80	1.15	46	184	0.70
pk	5.96	0.50	0.054	0.031	0.86	1.68	54	173	0.75
npk	6.32	0.52	0.056	0.070	0.76	1.86	52	156	0.86
Min. 5% sig. diff.	2.04	0.10	0.013	0.087	0.30	0.66	16	34	0.08

MEAN EFFECTS

Effect	Ash	Nitrogen	Phosphorus	Magnesium	Potassium	Calcium	Iron	Manganese	Rubber
N	–0.4625	0.0412	–0.00225	–0.0034	–0.12625	–0.0800	–3.25	–17.125*	0.0300
P	1.5475**	–0.0188	0.02200***	–0.0011	–0.00375	0.6500**	5.00	– 2.125	0.0600**
K	–0.2725	–0.0038	–0.00525	0.0056	0.11375	–0.2125	0	– 1.375	–0.0375
95% conf. limit	1.0205	0.0511	0.00636	0.0437	0.14883	0.3302	7.82	16.785	0.0384
99% conf. limit	1.5098	—	0.00942	—	—	0.4885	—	—	0.0568
99.9% conf. limit	—	—	0.01454	—	—	—	—	—	—

Table 9. Seaport Estate Trial

Concentration of nutrient elements in dried laminae and girth of trees at sixty inches measured in June 1956

TREATMENT MEANS

Treatment	Ash%	N%	P%	Mg%	K%	Ca%	Fe p.p.m.	Mn p.p.m.	Girth cm
$n_0p_0k_0$	3.68	3.34	0.16	0.17	0.65	0.72	99	66	77.8
$n_1p_0k_0$	3.54	3.58	0.18	0.17	0.62	0.68	83	92	78.6
$n_2p_0k_0$	3.12	3.74	0.18	0.18	0.68	0.52	94	44	77.6
$n_0p_1k_0$	4.72	3.22	0.26	0.14	0.80	1.06	107	93	78.6
$n_1p_1k_0$	4.28	3.40	0.22	0.13	0.76	0.92	98	64	80.7
$n_2p_1k_0$	3.82	3.40	0.19	0.12	0.58	0.86	98	104	74.8
$n_0p_0k_1$	4.35	3.18	0.16	0.16	0.96	0.71	98	85	77.4
$n_1p_0k_1$	3.74	3.56	0.16	0.12	1.02	0.53	94	51	77.6
$n_2p_0k_1$	3.46	3.68	0.17	0.15	0.92	0.50	92	50	81.4
$n_0p_1k_1$	4.56	3.42	0.23	0.16	0.95	0.83	88	78	81.0
$n_1p_1k_1$	4.17	3.52	0.21	0.13	0.86	0.82	92	99	78.7
$n_2p_1k_1$	4.14	3.64	0.20	0.12	0.87	0.79	94	86	79.8
Min. 5% sig. diff.	0.94	0.37	0.10	0.04	0.31	0.27	16	40	8.1

MEAN EFFECTS

Effect	Ash	Nitrogen	Phosphorus	Magnesium	Potassium	Calcium	Iron	Manganese	Girth
N (linear)	–0.692**	0.325**	–0.014	–0.014	–0.079	–0.159*	– 3.38	– 9.50	–0.250
95% conf. limit	0.470	0.182	0.049	0.018	0.158	0.135	7.86	19.95	4.061
99% conf. limit	0.663	0.257	—	—	—	—	—	—	—
N (quadratic)	0.096	–0.119	0.006	0.020	–0.039	0.019	8.62	– 1.74	–0.775
95% conf. limit	0.813	0.316	0.084	0.031	0.275	0.234	13.62	34.55	7.032
P	0.634**	–0.080	0.050*	–0.026**	–0.002	0.269***	2.84	23.00**	0.508
K	0.209	0.055	–0.010	–0.011	0.248**	–0.099	– 3.66	– 2.50	1.292
95% conf. limit	0.383	0.149	0.040	0.015	0.129	0.110	6.42	16.29	3.315
99% conf. limit	0.541	—	—	0.021	0.183	0.156	—	22.99	—
99.9% conf. limit	—	—	—	—	—	0.223	—	—	—

Table 10. Seaport Estate Trial

Concentration of nutrient elements in dried bark and yield of rubber in tahils per tree per tapping during September 1956 to January 1957

TREATMENT MEANS

Treatment	Ash%	N%	P%	Mg%	K%	Ca%	Fe p.p.m.	Mn p.p.m.	Rubber tahils
$n_0p_0k_0$	6.49	0.50	0.034	0.067	0.44	2.18	39	96	0.52
$n_1p_0k_0$	4.63	0.50	0.028	0.052	0.32	1.53	30	99	0.53
$n_2p_0k_0$	4.20	0.57	0.032	0.052	0.35	1.34	38	74	0.60
$n_0p_1k_0$	6.50	0.46	0.056	0.048	0.38	2.23	48	87	0.50
$n_1p_1k_0$	5.23	0.54	0.056	0.048	0.42	1.68	32	69	0.69
$n_2p_1k_0$	5.33	0.58	0.054	0.065	0.29	1.80	32	124	0.48
$n_0p_0k_1$	6.52	0.44	0.032	0.058	0.62	2.06	46	120	0.61
$n_1p_0k_1$	6.87	0.55	0.034	0.057	0.70	2.14	48	102	0.64
$n_2p_0k_1$	5.12	0.51	0.036	0.060	0.61	1.54	36	124	0.74
$n_0p_1k_1$	5.56	0.45	0.042	0.034	0.60	1.71	32	76	0.62
$n_1p_1k_1$	5.20	0.48	0.044	0.040	0.56	1.57	28	94	0.60
$n_2p_1k_1$	5.28	0.54	0.044	0.038	0.47	1.70	38	86	0.68
Min. 5% sig. diff.	2.74	0.10	0.014	0.024	0.28	0.90	30	58	0.21

MEAN EFFECTS

Effect	Ash	Nitrogen	Phosphorus	Magnesium	Potassium	Calcium	Iron	Manganese	Rubber
N (linear)	–1.285	0.090**	0.00037	0.002	–0.081	–0.450	– 5.00	– 5.25	0.065
95% conf. limit	1.372	0.051	0.00702	0.012	0.142	0.452	15.15	29.22	0.105
99% conf. limit	—	0.072	—	—	—	—	—	—	—
N (quadratic)	0.290	–0.023	0.00089	0.007	–0.056	0.182	8.00	2.25	–0.045
95% conf. limit	2.376	0.088	0.01215	0.021	0.245	0.782	26.24	50.60	0.182
P	–0.124	0	0.01650***	–0.012*	–0.055	–0.016	– 4.25	– 1.50	–0.013
K	0.359	–0.028	–0.00483	–0.008	0.227**	–0.004	1.75	0.17	0.097*
95% conf. limit	1.120	0.042	0.00573	0.010	0.116	0.369	12.37	23.86	0.086
99% conf. limit	—	—	0.00808	—	0.164	—	—	—	—
99.9% conf. limit	—	—	0.01154	—	—	—	—	—	—

November 1956. Girth, measured at 60 inches above the union in June 1956, did not show any significant response to fertiliser application. Yield of rubber per tree per tapping, averaged over the period September 1956 to January 1957, showed a significant mean effect attributable to fertilisers containing potassium. The mineral analyses of bark and laminae samples are given in Tables 9 and 10.

Both bark and lamina compositions reflected the effect of the addition of fertilisers containing potassium; the mean effect of this addition on potassium concentration in these tissues was significant. Thus the increase in yield of rubber on the addition of potassium fertilisers was accompanied by increases in the potassium contained in laminae and bark.

The inclusion of fertilisers containing phosphate in any treatment gave rise to a positive significant mean effect on the phosphorus concentration in both laminae and bark, but the effect was more strongly shown in terms of bark composition. Phosphate addition also significantly reduced the concentration of magnesium in both laminae and bark tissues. The application of phosphate fertilisers produced significant positive mean effects on the concentration of ash, calcium and manganese in the laminae but such effects were not recorded for the bark. Thus, as for the Wardieburn Estate trial, phosphate fertiliser addition affected the concentration of a greater number of elements in the laminae than in the bark; bark composition however, did show marked increases in phosphorus concentration wherever phosphate had been applied.

The addition of nitrogen fertilisers produced a significant increase in the nitrogen concentration of both bark and laminae; it also reduced the calcium concentration of the laminae but not of the bark.

Correlationships Between Compositions of Laminae and Bark

Significant correlationships were established between lamina and bark concentrations of phosphorus, potassium and manganese for each of the three manurial trials examined herein (Table 11); these correlationships were significant at the 1% level. Similar significant correlationships were also established in respect of nitrogen and calcium for samples taken from two out of the three trials. Significant correlationships were for ash and magnesium concentrations for samples taken from the Wardieburn Estate trial but none of the trials gave any indication of a significant correlation in respect of iron.

Table 11. Correlation Coefficients Between Concentrations of Nutrient Elements in Laminae and Bark

Trial	Ash	N	P	Mg	K	Ca	Fe	Mn
Field 31 (24 pairs of values)	0.204	0.125	0.538**	0.265	0.792***	0.723***	0.330	0.897***
Wardieburn Estate (16 pairs of values)	0.759***	0.689**	0.774***	0.923***	0.797***	0.868***	0.127	0.740**
Seaport Estate (24 pairs of values)	0.405	0.523**	0.660***	0.108	0.872***	0.198	–0.007	0.538**

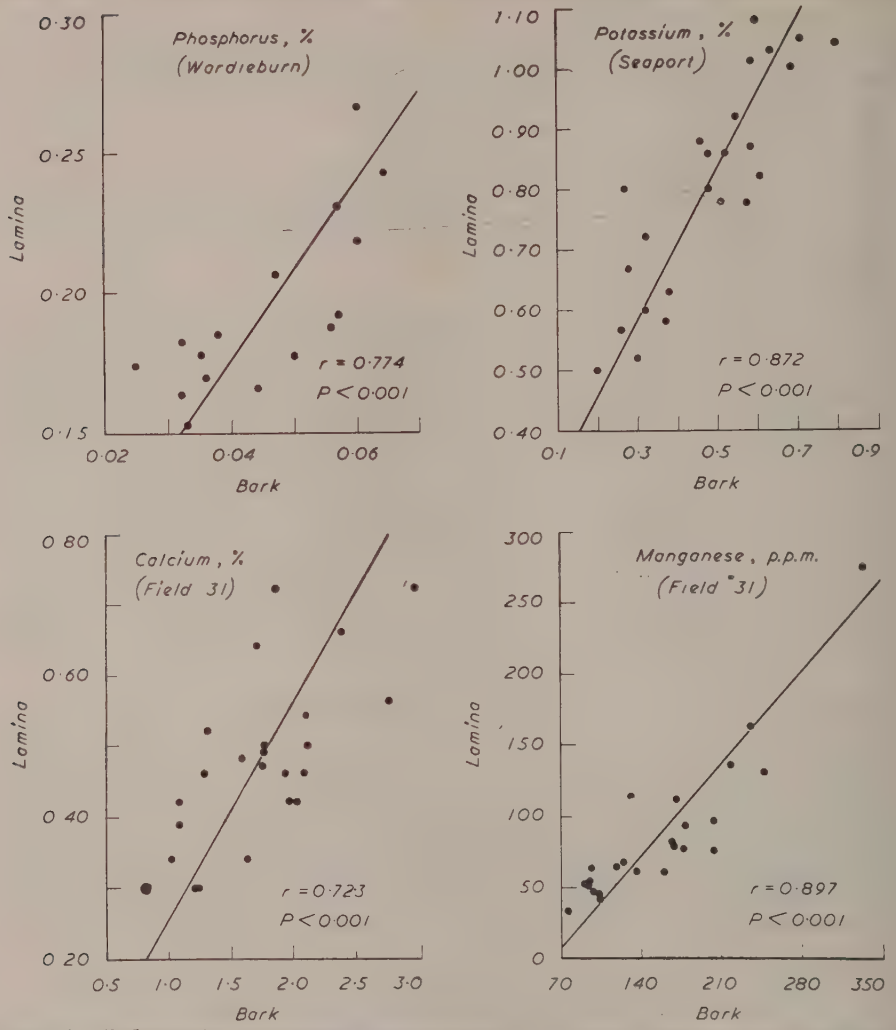

Figure 1. Relationships between concentrations of nutrient elements of bark and laminae. Concentrations expressed in terms of dried bark and lamina.

Some of these correlationships are demonstrated in *Figure 1* which also serves to illustrate that, for calcium and manganese the bark concentration values were usually of a higher order than those of laminae but for phosphorus the opposite was true. The concentrations of nitrogen, magnesium and potassium were smaller for the bark than for the laminae.

On the basis of the above findings it could be assumed that the concentrations of nitrogen, phosphorus, potassium, calcium and manganese in both bark and laminae could be regarded as capable of reflecting the same response to fertiliser addition but that the same could not, with confidence, be said in respect of magnesium.

GENERAL DISCUSSION

As indicated in the introduction to this paper, a satisfactory procedure is now available for the sampling of leaves of young hevea trees up to the one year old stage. The number of trees to be sampled per acre is a matter to be settled at the discretion and experience of the collector. A minimum unit of 5 trees has been mostly used and these have been usually located within half an acre; for large uniform soil areas the unit may be kept the same but the area increased. When leaves are removed according to storey position, about 40 leaves will be found to comprise one sample. For mature trees about 100 leaves are removed from a similar unit of trees, as described in the text. This larger number of leaves is taken in an attempt to balance the variability, according to crown position, found in mature trees. All recommendations regarding sampling are clearly arbitrary and made mainly to suit the convenience of the collector. It would undoubtedly be more efficient and accurate to double or treble the number of trees sampled and to reduce the number of leaves taken per tree. However, with a large number of samples to be taken this approach has been found to be impracticable in the field, owing to the long time required to take even one sample. Time may become an important limiting factor to be considered in any sampling as, based on the findings described in the early part of this paper, leaf samples should be taken between 06.00 to 10.00 hours. The collection of a large number of samples is preferred within this time limit by taking leaves from few trees, rather than to take a few samples derived from a large number of trees. Once a boy has climbed the tree to a selected spot it is hardly more trouble for the collector to take 20 leaves per tree from the cut down branches than it would be to collect about 3.

It was largely because of the unsatisfactory means of selection and collection of leaf samples from mature trees that the use of bark composition, as a means of determining the mineral nutrient status of trees, was considered. While it was never contemplated that this approach would excel the method of diagnosis based on leaf analysis it was nevertheless hoped that the analysis of easily collected bark samples would give a similar, but perhaps less sensitive index of nutritional status. This hope was partly realised and is discussed more fully below.

The concentrations of phosphorus, potassium and manganese in the laminae were closely correlated with the respective concentration found in the bark, for each of the manurial trials examined. Similar correlationships were also established for nitrogen and calcium for two out of the three trials considered. Therefore it seemed warranted to assume that when a response to fertiliser addition was obtained for laminae, in terms of concentration of any of the above elements, the bark also would show a similar response. This assumption was correct to a limited extent. Thus the addition of phosphate fertiliser in the Field 31 trial produced significant mean effects on the phosphorus concentrations of both laminae and bark. For the Wardieburn Estate trial, the same was true in regard to phosphorus and calcium concentration but not for effects on the manganese and iron concentrations, which were only given by the laminae. Similarly for the Seaport Estate trial, while both bark and lamina compositions showed responses to phosphate fertiliser in terms of phosphorus and magnesium concentrations the laminae also showed responses in terms of calcium and manganese concentrations.

Thus, for all trials, the phosphorus concentration of the bark as well as that of the laminae, reflected the addition of phosphate fertiliser but frequently the laminae composition showed effects which were not detectable in the bark. In Malaya a response, as measured in terms of girth and yield of rubber per tree, is frequently obtained as a result of the addition of phosphate fertiliser (Owen, Westgarth & Iyer 1957). See

Tables 5 to 8. Bark composition affords a ready means of assessing this response to phosphate fertiliser addition. For such a limited purpose, it seems credible that the bark may serve as a better index than the laminae; the bark composition may reflect the previous history and treatment given to the tree whereas the composition of the laminae can only mirror the outcome of these effects.

A response to potassium fertilisers, as measured by changes in the concentration of minerals in the laminae, was obtained for potassium, but only for one of the manurial trials examined (Seaport Estate). This same response was also reflected in the concentration of potassium found in the bark. As the potassium concentrations of laminae and bark were so closely correlated for all three trials and particularly in view of the effect obtained for the Seaport Estate trial it is considered that whenever laminae are likely to show responses, in terms of potassium concentration, to potassium fertilisers addition, bark will also respond similarly.

For nitrogen the evidence is less satisfactory, the correlationship coefficients, in respect of the nitrogen concentrations in bark and laminae, were inferior to those obtained for potassium and phosphorus. However, for the Seaport Estate trial, bark composition was as satisfactory an index (as measured by nitrogen concentration) as that of the laminae but for the Wardieburn Estate trial the mean effect of nitrogen fertiliser addition was shown only by the laminae.

These findings lead to the conclusion that bark composition may be used as a means of detecting responses to phosphate and potassium fertiliser additions but this may not always be so for nitrogen fertilisers. Further investigations in regard to the effect of the latter are required. By an extended application of bark sampling and analysis it should become possible to establish the normal level of potassium and phosphorus concentrations in the bark (and perhaps nitrogen also) and to relate them to yield performance. It is already clear from Tables 6, 8 and 10, that whenever a significant mean effect on yield is attributable to fertilisers containing phosphorus, or potassium, the bark composition mirrors this effect in terms of the concentrations of those elements. The ease of bark sampling is much in favour of its eventual adoption for routine evaluation of responses in manurial trials. The method of taking bark slivers can be altered to that of taking circular discs if this proves to be more amenable for routine purposes. The number of trees sampled must be varied according to the area being assessed. The advantages of the collection of bark samples as compared with the collection of leaf samples are obvious and need no further enumeration.

SUMMARY

An evaluation of the variation in the concentration of mineral nutrients in the laminae of young rubber plants during the day, showed that a significant diurnal variation occurred only in respect of magnesium; there were indications, but not proven, that a diurnal variation in iron concentration also occurred. Leaf sampling procedures are described and discussed in relation to diagnosis based on the composition of bark.

The effects of fertiliser addition on the mineral status of the tree were assessed, in three manurial trials, in terms of bark and lamina composition. It was concluded that bark composition offered a means of detecting responses to fertilisers containing phosphate, and potassium, almost as satisfactory as that of the lamina. Whenever a significant mean effect on yield of rubber produced per tree was attributable to the addition of phosphorus or potassium, the composition of the bark was found to mirror this effect in terms of the concentrations of those elements.

The concentrations of phosphorus, potassium and manganese in the lamina were closely correlated with the respective bark concentration; a similar relationship held for nitrogen and calcium in respect of samples taken from two out of the three trials examined. The concentration of iron in the lamina was not correlated with that found in the bark.

Soils Division
Rubber Research Institute of Malaya
Kuala Lumpur

May

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Cobalt: Effects on the Growth and Composition of Hevea

E. W. BOLLE-JONES and V. R. MALLIKARJUNESWARA

The supply of a minute amount (0.005 p.p.m.) of cobalt to young hevea seedlings grown in sand increased their height, girth and weight but made little difference to their nutrient element composition.

WITHOUT AN ADEQUATE SUPPLY OF COBALT sheep develop symptoms, sometimes referred to as enzootic marasmus or pining, and die if not resuscitated with either cobalt or vitamin B_{12} (MARSTON & LEE 1952, ANDERSON & ANDREWS 1952). Cobalt has also been demonstrated to be an essential element required by certain blue green algae (HOLM-HANSEN, GERLOFF & SKOOG 1954) and there seem to be apparent requirements for the element shown by green algae and other micro-organisms (HUTNER, PROVASOLI, SCHATZ & HASKINS 1950, DARKEN 1953). However, while it has been demonstrated that cobalt will materially increase the elongation of stem segments in the presence of sugars (MILLER 1954, THIMANN 1956) and can activate various enzyme systems (ANDERSON 1945), there is no conclusive proof that the element is essential to the growth of higher plants. HEWITT & BOLLE-JONES (1952) failed to produce evidence that cobalt was essential for the growth of tomato, lettuce or sugar beet and the beneficial effect claimed for cobalt on cacao (MASKELL, EVANS & MURRAY 1953) was not supported by convincing chemical or other evidence.

The evidence summarised below shows that a minute amount of cobalt supplied in nutrient solution to rubber plants grown in sand significantly improved growth. It shows that cobalt in small amounts has a beneficial effect on the growth of the rubber plant but that larger concentrations may depress growth; this knowledge in itself does not prove cobalt to be an indispensable nutrient element.

EXPERIMENTAL METHODS

Hevea brasiliensis seedlings, clone Prang Besar 86, were grown in purified river sand at three different levels of cobalt supply. These were:

Co_1 ... a nil level at which no cobalt was deliberately supplied. However, it was known from the chemical analysis of the waters used to prepare the nutrient solution that contamination occurred, to the extent of 0.000015 p.p.m. of cobalt.

Co_2 ... a normal level at which 0.005 p.p.m. of cobalt was supplied in the nutrient; this concentration was sufficient to maintain normal healthy growth.

Co_3 ... a high level at which 0.25 p.p.m. of cobalt was given; this concentration was anticipated to be well in excess of the plant's supposed requirement for cobalt.

The sand was contained in pyrex glass, four-gallon capacity, freely drained vessels of which there were four per treatment replicate; each treatment was carried out in duplicate, thus making a total of 24 containers.

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The sand (particle size 0.5 to 1.5 mm) was purified by washing batches of 420 lb with 50 litres of a hot (100°C) mixture of 17% (w/v) hydrochloric and 1% (w/v) oxalic acids for seven hours; this procedure was thrice repeated for each batch and on each occasion a fresh acid charge was used. After washing, the sand was transferred to pyrex glass vessels each of which had been steamed for at least twenty minutes. The sand was restored to neutrality by continual leaching with glass distilled water (conductivity 1.3 mho) and the purified basal nutrient subsequently used in the experiment itself. At the beginning of the experiment glass distilled water was used to prepare the nutrient solutions but later rainwater, which had been demineralised by passage through ion exchange resin units and possessed a conductivity of about 4 mho, was also used. The concentrations of cobalt in both glass distilled water and demineralised rainwater were similar and in the region of 0.000015 p.p.m. of cobalt.

The same volume of respective nutrient was given to each vessel in all treatments. The one difference between treatments was the variation in the cobalt level. The composition of the basal nutrient supplied was (in milligram equivalents per litre): NO_3^- 5, NH_4^+ 3, SO_4^{--} 7.5, PO_4^{---} 3, K^+ 3, Mg^{++} 2.5, Ca^{++} 4, Na^+ 1, Fe^{+++} 1, Mn^{++} 0.02, BO_3^{---} 0.033, Cu^{++} 0.002, Zn^{++} 0.002, Mo(hexavalent) 0.001, Al^{+++} 0.001, Ni^{++} 0.0002, Ga^{+++} 0.0002. Nutrients were freed of cobalt in the following fashion.

The macronutrient element ions were supplied as the following salts: $(NH_4)_2SO_4$, KNO_3, K_2SO_4, $NaH_2PO_4.2H_2O$, $MgSO_4.7H_2O$ and $Ca(NO_3)_2$. With the exception of potassium nitrate and potassium sulphate, a nearly saturated solution of each salt was prepared and treated when warm with a solution of alpha-nitroso beta-naphthol (0.12% w/v) in aqueous sodium hydroxide (0.05% w/v). According to the purity of the salt, 15 to 40 ml of this reagent per kilogram of salt were used. After cooling, the salt solution was extracted with redistilled chloroform to remove most of the cobalt alpha-nitroso beta-naphthol complex together with any other heavy metal which may have been present. Finally, 0.5 to 1.0 gram of activated carbon was added per litre of salt solution which was warmed, cooled and slowly filtered without applied suction. The clear filtrate was made up to any desired volume.

The potassium nitrate and potassium sulphate salts were purified by different and more laborious methods. To a nearly saturated solution of the salt 5 ml of a 0.1% (w/v) aqueous solution of nitroso R salt and 10 ml of a 50% (w/v) aqueous solution of sodium acetate were added, per kilogram of salt. The cobalt nitroso R salt complex and the excess of organic reagent were adsorbed on activated carbon (0.5 to 1.0 gm of carbon per litre of solution) and a clear filtrate was subsequently obtained. The clear potassium nitrate filtrate was warmed and subjected to the alpha-nitroso beta-naphthol extraction procedure and subsequently crystallised from solution by the addition of an equal volume of ethanol. The potassium sulphate salt was crystallised directly from the clear filtrate after the addition of ethanol and this recrystallisation was twice repeated. There were no grounds for belief that the nitroso R salt purification process was less efficacious than the alpha-nitroso beta-naphthol method but as it required the addition of sodium acetate, which was not subsequently removed, the other process was preferred. In terms of the unpurified solid salts, the following cobalt concentrations were recorded (as p.p.m.): $(NH_4)_2SO_4$ 0.67; KNO_3 0.40; K_2SO_4 0.07; $MgSO_4,7H_2O$ 0.55; $Ca(NO_3)_2$ 0.47.

Manganese was supplied as the sulphate, which was purified by the alpha-nitroso beta-naphthol procedure as described above. The remainder of the salts used to supply the micronutrient elements were purified by simple recrystallisation from water. Iron was supplied as ferric citrate which was prepared and cleansed of cobalt in the following manner. Ferrous sulphate ($FeSO_4.7H_2O$), which was found to contain 3.0 p.p.m. of cobalt, was oxidised to ferric sulphate with redistilled nitric acid and the ferric ion

transferred to ether by extraction from a 6N hydrochloric acid medium. The ferric chloride, in the ether layer, was taken up with glass distilled water and a further extraction by ether in the presence of 6N hydrochloric acid was carried out; this process was repeated. The ferric chloride was then free of cobalt ($CoCl_2$ is insoluble in ether). Pure ammonium hydroxide was prepared by redistillation from 25% aqueous ammonia and the absorption of the ammonia gas in glass distilled water. Cobalt free ammonium citrate was prepared by the admixture of equivalent quantities of citric acid and aqueous ammonia and the subsequent purification of the solution mixture according to the alpha-nitroso beta-naphthol procedure. Ferric hydroxide was precipitated from a known volume of the cobalt free ferric chloride by the addition of purified ammonium hydroxide; the ferric hydroxide was filtered off and washed with glass distilled water. The ferric hydroxide was transferred to a pyrex glass flask. A stoichiometric equivalent of ammonium citrate, free of cobalt, was added and the solution mixture boiled until all the hydroxide went into solution and ammonia evolution ceased.

Utmost care was taken throughout to prevent contamination of sand, pots or nutrients. Pyrex glassware was used to prepare or supply the nutrient solutions; before being used it was washed with concentrated nitric acid and glass distilled water, was thoroughly steamed and again washed with nitric acid.

Eighteen clone Prang Besar 86 seeds, each weighing more than 4.5 grams and well washed with glass distilled water, were sown in each pyrex glass vessel on 8 December 1955. Each glass vessel or 'urn liner' possessed a central drainage hole which was covered by an inverted pyrex watchglass; each was also painted black on the outside and wrapped round with light brown paper. After the preliminary thinning out, complete plants were removed for analysis on the following dates: 16 February, 24 April, 19 June, 8 August, 20 November. At the earlier samplings a minimum of two plants was removed from each vessel until at the final, November, sampling one plant only remained. Plants taken from vessels within a treatment replicate were bulked together and segregated into laminae, stems and petioles, and roots, each of which were analysed separately. Most of the methods of analysis have been described elsewhere (Bolle-Jones 1954, Bolle-Jones, Mallikarjuneswara & Ratnasingam 1957). Cobalt was determined according to the following procedure. Five to twenty grams of material, according to the cobalt concentration of the sample, were dry ashed and dissolved in nitric acid (1:1); the acid solution was evaporated to dryness and the residue taken up in phosphoric acid (1:49). Thereafter the procedure for the absorptiometric measurement of the cobalt nitroso R salt complex was similar to that described by Saltzman (1955).

Apart from the main experiment described above, some pyrex glass vessels were allocated for the growing of tomato plants at the Co_1 and Co_2 levels in a pilot scale trial. The tomato seeds, extracted from a locally purchased fruit of unknown origin, were thoroughly washed and air dried before being sown on 21 December 1955. Imported seeds of known variety could not be used as they were sold in packets containing cobalt stained silica gel. The plants received a similar nutrient solution to that given to the rubber plants except that the NO_3^-, PO_4^{---} and Ca^{++} concentrations were increased to 9, 4.5 and 8 milligram equivalents/litre, respectively. In all other respects the plants received the same treatment as the rubber seedlings grown in the major experiment.

RESULTS

Effects of Cobalt Supply on Growth

The rubber seedlings did not show any leaf symptoms which could be attributed to a deficiency or toxicity of cobalt but other effects on height, girth and weight, were

recorded. Height measurements were taken, at least, at monthly intervals, and regression equations to fit these measurements were calculated to be as follows, where x = number of days from sowing and y = height of plant (cm). The plant height at the time of sowing, nominally zero centimetres, was taken into account in the computation.

Co_1	$y = -11.1$	+	$0.82\ x$
	(± 2.16)		(± 0.022)
Co_2	$y = -17.7$	+	$0.97\ x$
	(± 2.16)		(± 0.022)
Co_3	$y = -12.9$	+	$0.88\ x$
	(± 2.45)		(± 0.025)

The differences between the regression coefficients were shown to be significant at the 0.1% level (Table 1); the slopes of the regression lines were significantly different and the slope of the Co_1 line was significantly less than that of the Co_2 line. The Co_1 plants were shorter than those of the Co_2 treatment (*Figure 1*) whereas the Co_3 plants were intermediate in height between the Co_1 and Co_2 plants. These points are illustrated by the height measurements taken in early August 1956; the following mean values (cm) were recorded: Co_1 = 183, Co_2 = 215, Co_3 = 193 (minimum 5% significant difference = 24).

The stem girth of the rubber seedlings was measured at a height of 7 centimetres above sand level, from May 1956 onwards, at monthly intervals. The measurements clearly showed that the girths of the Co_1 plants were consistently smaller than those of the Co_2 plants and that the Co_3 values ranged somewhere between. The graph relating girth to time of measurement was not linear and a transformation to linearity using log(1 + girth) values was used. The regression equations so obtained, where x = number of days from sowing and y = $\log_{10}(1 + \text{girth})$ with girth measured in centimetres, were:

Co_1	$y = 0.248$	+	$0.00220\ x$
	(± 0.0026)		(± 0.000045)
Co_2	$y = 0.248$	+	$0.00237\ x$
	(± 0.0037)		(± 0.000064)
Co^3	$y = 0.208$	+	$0.00244\ x$
	(± 0.0044)		(± 0.000077)

TABLE 1. SIMPLE REGRESSION ANALYSIS OF RUBBER SEEDLING HEIGHT MEASUREMENTS

Measurements recorded (cm) for each treatment replicate, at thirteen different times from date of sowing

Variation	d.f.	Mean squares
Common regression	1	619091.84***
Difference between regression coefficients	2	1501.34***
Sum of individual regressions	3	207364.84***
Sum of residuals	75	138.29
Total	78	

***0.1% sig.

Figure 1. Comparison of heights of eleven months old rubber seedlings grown at the Co_1 (right) and Co_2 (left) levels. Each scale gradation is equivalent to three inches.

The differences between the regression coefficients were shown to be significant at the 5% level (Table 2). The differences between the slopes of values for the three levels of cobalt were significant; the slope of Co_1 line was significantly less than the slope of each of the other two levels but a difference in slope between the Co_2 and Co_3 lines was not established. These points are reflected in the treatment mean for each level for girth measurements (cm) taken in mid-November 1956: Co_1 = 8.80, Co_2 = 9.96, Co_3 = 9.50; minimum 5% significant difference = 0.50.

The total dry weight per Co_1 plant was consistently smaller than that for the Co_2 plant, for the four later samplings; the Co_3 values were usually closer to and slightly smaller than those of the Co_2 plants. A logarithmic transformation of the data relating dry weight to time showed that the slopes of the regression lines did not differ significantly between cobalt levels and that one regression line could represent the relationship between the logarithm of dry weight and time of sampling for all three levels of cobalt supply. An analysis of variance of the total dry weight per plant values recorded at the final (November) sampling showed that the effect of cobalt level was significant; depending upon whether Co_1 and Co_2 only, or all three treatments were analysed, the effect of cobalt

Table 2. Regression Analysis of Rubber Seedling Stem Girth Measurements

Measurements recorded for each treatment replicate once every month, over the last eight month period of the experiment. Measurements (cm) were transformed to log(1 + girth) function before analysis.

Variation	d.f.	Mean squares
Common regression	1	0.86***
Difference between regression coefficients	2	0.0008*
Sum of individual regressions	3	0.29***
Sum of residuals	42	0.0002
Total	45	

*5% sig. ***0.1% sig.

was significant at the 1% and 5% levels respectively (Table 3). It was again quite clear that the Co_1 plants were inferior to the Co_2 plants which were also bigger but not significantly so, than the Co_3 plants.

Table 3. Variance Analyses of Dry Weight per Plant Values for Rubber Seedlings Removed at the November Sampling

Analysis of values (gm) carried out with respect to all treatments (i) or with regard to Co_1 and Co_2 treatments only (ii).

Variation	(i) Co_1, Co_2 and Co_3		(ii) Co_1 and Co_2 only	
	d.f.	Mean squares	d.f.	Mean squares
Cobalt levels	2	19756.0*	1	37392.0**
Replication	1	295.0	1	99.4
Error	2	864.8	1	2.6
Total	5		3	
S.e. of cobalt mean		±20.8		±1.2
Min. 5% sig. diff.		126.5		20.6
Cobalt means (gm)	Co_1 = 597.9, Co_2 = 791.3, Co_3 = 734.5			

*5% sig. **1% sig.

The dry weight of roots per plant reflected the effect of cobalt level more sensitively than the total dry weight of plant (shoot + root). After transformation to linearity using a log(1 + dry weight of roots per plant) function the following regression equations were arrived at where x = number of days from sowing and y = $\log_{10}$(1 + dry weight of roots per plant in grams):

Co_1 $y = -0.282 + 0.00694\ x$ (± 0.027) (± 0.00028)

Co_2 $y = -0.340 + 0.00773\ x$ (± 0.021) (± 0.00022)

Co_3 $y = -0.369 + 0.00754\ x$ (± 0.025) (± 0.00026)

The slopes of these lines were different, but only at the 10% level of significance when all three were considered; but if consideration was confined to the Co_1 and Co_2 lines, the difference became significant at the 5% level (Table 4). The weight of roots per Co_1 plant was less than for a Co_2 plant (*Figure 2*) at the four later samplings; the difference between the Co_2 and Co_3 root weights was not always significant. The mean root weights (gm) per plant recorded at the last (November) sampling were: Co_1 = 123, Co_2 = 217, Co_3 = 193 (minimum 5% significant difference = 39).

The graphs relating height, girth and root dry weight values to time (from date of sowing) have been presented elsewhere in a brief preview (Bolle-Jones & Mallikarjuneswara 1957) of the present findings and are not included here. The above evidence, which relates to the effect of cobalt supply on various aspects of growth of

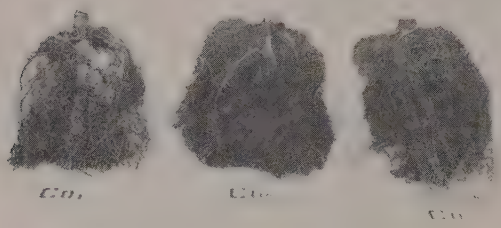

Figure 2. Roots of rubber seedlings grown at the Co_1, Co_2 and Co_3 levels as observed at the November sampling. Note the greater mass of roots of the Co_2 plant as compared with the Co_1 plant and the slight reduction in root production of the Co_3 plant as compared with the Co_2 plant.

the rubber seedling, leaves little room to doubt that a small trace of added cobalt brings about an improvement in growth.

The tomato seedlings, grown in the pilot trial alongside the rubber seedlings, also did not show any visual symptom attributable to a lack of cobalt. However, beneficial effects of cobalt on the dry weights of shoot and fruit per plant were detectable (Table 5), thus confirming the influence of cobalt on growth as observed in the rubber seedlings.

Effects of Cobalt Supply on Chemical Composition of Plant

At the end of the experiment each Co_1 plant contained 9.5 micrograms of cobalt whereas each Co_2 and Co_3 plant contained 267 and 9490 micrograms of cobalt, res-

Table 4. Regression Analysis of Dry Weight of Roots per Plant for Rubber Seedlings Removed at each of the Five Samplings

Values, which were transformed to their log(1 + dry weight of roots in grams) function before analysis, were analysed with respect to all treatments (i) or with regard to Co_1 and Co_2 treatments only (ii).

Variation	(i) Co_1, Co_2 and Co_3		(ii) Co_1 and Co_2 only	
	d.f.	Mean squares	d.f.	Mean squares
Common regression	1	14.7***	1	9.6***
Difference between regression coefficients	2	0.02+	1	0.03*
Sum of individual regressions	3	4.9***	2	4.8***
Sum of residuals	24	0.006	16	0.006
Total	27		18	

+10% sig. *5% sig. **1% sig. ***0.1% sig.

pectively. It was obvious that the Co_1 plants received an illegitimate source of cobalt supply despite the stringent precautions taken by us against contamination of sand and nutrients. This contamination was of the order of 8 to 9 micrograms of cobalt per plant as the Prang Besar rubber seeds used in this experiment were found to contain an average of 0.8 micrograms of cobalt each.

Table 5. The Dry Weights of Shoots, Roots and Fruits of Tomato Plants of Different Ages Grown at Different Cobalt Levels

Expressed as gm dry weight per plant

Days from sowing	Cobalt level	Shoot	Root	Fruit
40	Co_1	0.15	0.04	—
	Co_2	0.21	0.10	—
47	Co_1	1.23	0.22	—
	Co_2	1.34	0.23	—
91	Co_1	33.1	4.2	—
	Co_2	33.0	5.2	—
111	Co_1	92.2	5.2	3.2
	Co_2	103.7	5.1	6.1
167	Co_1	197.0	11.8	35.1
	Co_2	321.8	14.5	63.3

The concentrations of cobalt, rubber, nitrogen, phosphorus, magnesium, potassium, calcium, manganese and iron were determined in the laminae, stems and petioles and roots on each of the five samplings; the chlorophyll concentration of the laminae was also estimated. Surprisingly little, and not significant, variation due to the level of cobalt supply was noted for rubber, nitrogen, phosphorus, magnesium, potassium and chlorophyll. Table 6 gives the results obtained for the final (November) sampling. For iron and calcium in roots and for manganese in laminae evidence of a cobalt effect

Table 6. Concentrations in Laminae, Stems and Petioles, and Roots, of Rubber Seedlings

Concentrations of rubber, chlorophyll, nitrogen, phosphorus, magnesium, potassium and calcium given as % of dry matter; of iron, manganese and cobalt given as p.p.m. of dry matter. November 1956 sampling.

Part of plant	Level of cobalt	Co	Rubber	Chloro-phyll	N	P	Mg	K	Ca	Fe	Mn
Laminae	Co_1	0.02	0.72	1.02	3.3	0.34	0.27	1.1	0.64	162	70
	Co_2	0.36	0.81	0.89	3.5	0.38	0.28	1.1	0.64	184	77
	Co_3	4.41	0.74	0.84	3.3	0.34	0.32	1.0	0.68	174	57
Stems & petioles	Co_1	0.01	0.34	—	0.9	0.18	0.20	0.8	0.38	40	31
	Co_2	0.08	0.32	—	0.8	0.22	0.22	0.7	0.36	26	30
	Co_3	0.90	0.38	—	0.8	0.18	0.20	0.7	0.35	37	25
Roots	Co_1	0.03	0.44	—	1.1	0.34	0.16	1.1	0.14	861	16
	Co_2	0.83	0.42	—	1.3	0.34	0.15	1.4	0.19	1314	18
	Co_3	44.03	0.38	—	1.2	0.38	0.16	1.4	0.14	1296	15

was obtained; these are examined below, together with the variation in the concentration of cobalt in the tissues.

The concentration of cobalt was highest in roots and least in stems and petioles of the rubber seedlings; the concentration of cobalt in the laminae of the Co_2 plants was about one half of that in the roots and was slightly greater than that of the stems and petioles (Table 7). The concentration of cobalt in the laminae of the Co_1 plants, about 0.02 p.p.m., was too high to permit the development of symptoms, if there are any, associated with cobalt deficiency. The rubber seedling roots, which gave the best indication of cobalt status, sometimes contained as much as 44 p.p.m. of cobalt in the Co_3 plants as compared with the corresponding 4.4 and 0.9 p.p.m. for the laminae and stems and petioles, respectively.

Table 7. Concentrations of Cobalt in Laminae, Stems and Petioles and Roots of Rubber Seedlings

Expressed as p.p.m. of dry matter

Days from sowing	Cobalt level	Laminae	Stems & petioles	Roots
70 (Feb.)	Co_1	0.06	0.10	0.18
	Co_2	0.20	0.18	0.75
	Co_3	4.30	3.46	21.99
138 (April)	Co_1	0.01	0.005	0.05
	Co_2	0.27	0.19	0.56
	Co_3	6.55	4.76	30.14
194 (June)	Co_1	0.02	0.01	0.03
	Co_2	0.25	0.13	0.54
	Co_3	6.35	2.73	33.98
244 (Aug.)	Co_1	0.03	0.01	0.04
	Co_2	0.30	0.16	0.51
	Co_3	6.36	1.41	24.24
348 (Nov.)	Co_1	0.02	0.01	0.03
	Co_2	0.36	0.08	0.83
	Co_3	4.41	0.90	44.03

The concentration gradient of cobalt within the Co_2 tomato plants was different from that observed in the rubber seedlings as higher concentrations of cobalt were found in the stems and petioles than in the laminae. Five months old tomato plants showed the following concentrations of cobalt in the different tissues (as p.p.m. of dry matter).

	Co_1 plants	Co_2 plants
Laminae	0.03	0.17
Petioles	0.02	0.23
Stems	0.04	0.34
Roots	0.14	1.55

The concentration of calcium in the roots of the Co_2 rubber plants was usually higher than for those of the Co_3 for each sampling, but the difference between them was significant only for the last two samplings (Table 8). For the last sampling, also, the calcium value for the Co_1 roots was significantly less than for the Co_2 plants.

As with calcium, the effect of cobalt on the concentration of iron within the rubber seedling roots did not become consistent until the June and later samplings. The last sampling showed that the Co_1 plants possessed a significantly smaller iron concentration than those of the Co_2 plants (Table 8). The concentration of iron in the Co_3 roots was usually of the same order as for the Co_2 roots and a significant difference between the two was not established.

There were indications for the later samplings that the concentration of manganese in the Co_3 laminae was less than found for the Co_2 rubber plants; but this point was only established with confidence for the August sampling (Table 8).

Table 8. Concentrations of Calcium and Iron in Roots and of Manganese in Laminae of Rubber Seedlings

Expressed as % (Ca) or p.p.m. (Fe and Mn) of dry matter

Days from sowing	Cobalt level	Calcium % in dry roots	Iron p.p.m. in dry roots	Manganese p.p.m. in dry laminae
194 (June)	Co_1	0.17	450	76
	Co_2	0.18 (±0.013)	700 (±126)	77 (±5.2)
	Co_3	0.18	768	60
Min. 5% sig. diff.		0.08	770	32
244 (Aug.)	Co_1	0.16	553	61
	Co_2	0.17 (±0.003)	657 (±57)	70 (±1.5)
	Co_3	0.14	650	58
Min. 5% sig. diff.		0.02	345	9
348 (Nov.)	Co_1	0.14	861	70
	Co_2	0.19 (±0.003)	1314 (±45)	77 (±5.7)
	Co_3	0.14	1296	57
Min. 5% sig. diff.		0.02	276	35

DISCUSSION

The results clearly showed that traces of added cobalt were beneficial to the growth of rubber (*Figures 1* and 2) and tomato seedlings. Nevertheless, despite growth differences, cobalt level did not have any marked effect on the accumulation of rubber, nitrogen, phosphorus, magnesium, potassium and chlorophyll in the tissues (Table 6). It has been shown for many plants that an excess of cobalt will bring about iron deficiency chlorosis (Brown 1956). It seemed that the Co_3 level (0.25 p.p.m. Co) employed by us was too low to bring about such an effect although it was capable of reducing growth below that obtained at the Co_2 level. There were no indications of a severe chlorosis or a marked diminution of the iron concentration in laminae of plants grown at the Co_3 level. The only chemical data which could be interpreted as a sign of cobalt toxicity were the reductions of the concentrations of manganese in the

laminae and of calcium in roots at the later samplings, although these reductions were not always significant. The effect of cobalt level on the concentration of manganese in the laminae may be reconciled with reports that manganese deficiency symptoms have been observed in other crops as a result of an excessive cobalt supply (Hewitt 1953) and that manganese addition may alleviate cobalt toxicity effect (Ahmed & Twyman 1953).

Slender evidence was obtained, for the later samplings, which indicated that a lack of cobalt supply decreased the concentrations of calcium and iron in the roots. These tentative findings together with the growth effects, listed previously, comprise the effects of cobalt deficiency as detected by us. To our knowledge this is the first occasion on which it has been convincingly demonstrated that a deficiency of cobalt will reduce the total dry weight, height and stem girth of a plant. To explain the relatively well marked growth effects and lack of marked effects on chemical composition a suggestion made by Galston & Siegel (1954) seems attractive. It is that the cobaltous ion is capable of either reducing peroxigenesis or decomposing the peroxides as they are formed before they can be utilised in peroxidative reactions; this would curtail the destruction of indole acetic acid and allow of greater extension and other growth effects.

The evidence presented here does not constitute proof of the essentiality of cobalt for the growth and wellbeing of the higher plant. However, it is credible that with prolonged growth a perennial crop, such as the rubber tree, would soon show signs of cobalt deficiency if stringent cultural precautions against contamination were continued. On the basis of the data presented in Table 7 and with the knowledge that no visual symptoms of cobalt deficiency were produced in our experiment, it can be surmised that if rubber seedlings were to develop symptoms of cobalt deficiency the concentrations of cobalt in the laminae, stems and roots would be less than 0.01, 0.005 and 0.02 p.p.m. of cobalt (in terms of dried tissue), respectively.

SUMMARY

Rubber seedlings, in pot sand culture, were supplied with a very low, normal and high level of cobalt. Cobalt was eliminated from the nutrient solution by a new nutrient purification procedure. Seedlings deprived of cobalt did not show any leaf symptoms but were shorter, of less girth and of lighter weight than those given a normal level of cobalt supply. Tomato plants, also grown under similar conditions, showed a decreased yield of fruit and of dry weight of plant as a result of restricted cobalt supply. A deficient supply of cobalt (not exceeding 0.00002 p.p.m. Co) sometimes decreased the concentrations of calcium and iron in the roots but did not affect the concentrations of any of the large number of other nutrient elements examined, in the laminae, stems and petioles or roots.

A greater concentration of cobalt occurred in the roots of rubber seedlings than in the laminae which in turn showed a greater concentration than the stems and petioles. The concentration of cobalt in the roots may therefore provide the best and most easily determined index of cobalt status in the plant. A high level (0.25 p.p.m.) of cobalt in the nutrient tended to diminish growth below that obtained at the normal level (0.005 p.p.m.) of cobalt supply but no severe cobalt toxicity effects were observed.

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Soils Division
Rubber Research Institute of Malaya
Kuala Lumpur

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Molybdenum: Effects on the Growth and Composition of Hevea

E. W. BOLLE-JONES

Hevea seedlings were grown in sand at various supply levels of molybdenum, sulphate and calcium, or under molybdenum deficiency conditions with nitrate or nitrate and ammonium ions as a source of nitrogen. Effects were noted of molybdenum deficiency, which varied according to the levels of other applied nutrients, on growth and chemical composition. The deficiency may occur in Malaya but may be unaccompanied by well defined foliar symptoms.

THE IMPORTANCE OF MOLYBDENUM, as a plant nutrient, has become well established in recent years and spectacular crop yield responses to the addition of such small amounts as one ounce of molybdenum per acre are not unusual (ANDERSON 1956). The acidic nature and low lime status of local soils make it probable that molybdenum deficiency of both rubber trees and of leguminous cover plants occurs in Malaya. The occurrence of molybdenum deficiency has not been established with confidence although its presence is suspected in Johore and a claim has been made (SANDISON & HANLY 1954) of growth responses, to added molybdenum, by leguminous plants grown in that area. The research programme of the Soils Division of this Institute includes the artificial production, identification and cataloguing of the symptoms of each mineral nutrient deficiency; some of the results of these investigations were previously reported (BOLLE-JONES 1956a). An investigation of the effects of molybdenum deficiency on the growth and composition of *Hevea brasiliensis* comprised part of this programme. It was hoped that a description of the visual symptoms of molybdenum deficiency would facilitate the detection of the field occurrence of the deficiency and lead to remedial action.

The first part of this paper deals with an attempt to produce symptoms of molybdenum deficiency in rubber plants which were grown in sand and supplied with nitrogen either as nitrate alone or as a mixture of nitrate and ammonium salts. It was suspected that the requirement for molybdenum would be lower for plants supplied with nitrogen as ammonium salts than if given nitrate salts (HEWITT & MCCREADY 1956). It was also anticipated that the rubber plant would exhibit iron deficiency effects and not grow well on a high-nitrate source of nitrogen. It was therefore desirable to attempt to produce molybdenum deficiency in the absence of such a complicating factor and, for this reason, some of the rubber plants were supplied with a well tried nutrient solution which contained a large proportion of ammonium ions even though it was known that by doing so the requirement for molybdenum would probably be considerably reduced. Other plants were supplied with nitrogen as nitrate salts and were compared with those provided with ammonium ions. The experiment, although producing notable growth effects according to molybdenum status, was not entirely successful as the nature of the molybdenum deficiency symptoms was not established with certainty. A complicated syndrome arising from iron deficiency effects in the nitrate fed plants interfered with the interpretation of the visual responses while the cultural technique did not appear good enough to permit the appearance of molybdenum deficiency symptoms in plants fed with nitrogen, as ammonium salts. About

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one and a half years later another attempt was made to produce molybdenum deficiency in plants grown in a similarly designed experiment but with the modification that iron was supplied not as citrate but as the chelated ferric form of disodium ethylene-diamine-tetra-acetate. Unfortunately the use of this chelated form of iron induced zinc deficiency effects and had to be abandoned in favour of ferric citrate. A few of the preliminary results of this experiment are included in the present paper and serve to confirm the general conclusion that if nitrogen was partially supplied in the ammonium form the apparent requirement for molybdenum was much diminished and symptoms of the deficiency could not be readily produced.

The molybdenum uptake of plants may be governed by the amount of sulphate supplied to the roots (Stout, Meagher, Pearson & Johnson 1951); sulphur deficient plants possess a higher concentration of molybdenum in their laminae than do healthy plants (Bolle-Jones 1956b). As, therefore, the accumulation of molybdenum by plants is affected by their sulphate status and as molybdenum is made more readily available to plants by the addition of lime (Anderson 1956) it was decided to investigate the effects and interactions of molybdenum, sulphate and calcium on the growth of the rubber plant. While there was evidence that sulphate ions may directly influence molybdenum uptake by roots and so affect the concentration of molybdenum in the plant there was no indication of any effect of (nutrient) calcium ions on the concentration of molybdenum within the plant. Both sulphur and calcium are noteworthy as mineral nutrients required by *Hevea brasiliensis* in relatively small amounts. Neither sulphur nor calcium deficiency has been authoritatively reported to occur in Malaya although a deficiency of calcium might well be expected in crops grown on the strongly acid Malayan soils. The factorial experiment, designed to investigate the interrelationship between molybdenum, sulphate and calcium, showed that large differences in growth could often occur between treatments without the concomitant development of visual symptoms of any deficiency. Symptoms, believed to be those of molybdenum deficiency, were also observed and found to be more severe as the supply of sulphate to the plants increased. The relative importance of the three nutrient elements in the nutrition of the plant was well illustrated.

Molybdenum Deficiency Effects as Affected by Source of Nitrogen Supply

EXPERIMENTAL

Tjirandji 1 selfed seedlings were grown in purified sand contained in pyrex glass vessels with and without molybdenum and at different levels of nitrate and ammonium supply. Each of the four nutrient treatments was carried out in duplicate and consisted of the following levels of molybdenum, nitrate nitrogen, and ammonium nitrogen (in milligram equivalents per litre).

	Mo (hexavalent)	NO_3^-	NH_4^+
– Mo(N)	0	8	0
Complete(N)	0.001	8	0
– Mo(Am)	0	5	3
Complete(Am)	0.001	5	3

The pyrex glass vessels used to contain the sand were of a two gallon capacity and were thoroughly acid washed and steamed before use. The central drainage hole of each freely drained vessel was covered by a thin glass wool layer which was overlain by an inverted watchglass; both glass wool and watchglass were thoroughly washed with hot, constant-boiling, hydrochloric acid.

The sand (particle size 0.5 to 1.5 mm) was purified by three successive hot washings with a mixture of hydrochloric (14% w/v) and oxalic (1% w/v) acids; each washing lasted seven hours and required a fresh acid charge. The sand was subsequently restored to neutrality by leaching with a molybdenum-free, potassium and sodium nitrate solution until immediately prior to the commencement of the experiment, when the respective nutrients were applied.

Water distilled from, and condensed in, pyrex glass stills was used to prepare the respective nutrient which possessed a basal composition of (in milligram equivalents per litre) SO_4^{--} 2.5 (N type) 7.5 (Am type), PO_4^{---} 3, K^+ 3, Mg^{++} 2.5, Ca^{++} 4, Na^+ 2 (N type) 1 (Am type), Fe^{+++} 1, Mn^{++} 0.02, BO_3^{---} 0.033, Cu^{++} 0.002, Zn^{++} 0.002, Al^{+++} 0.001, Ni^{++} 0.0002, Co^{++} 0.0002, Ga^{+++} 0.0002.

The salts of the macronutrient elements were cleansed of molybdenum by the coprecipitation of the molybdenum contaminant with copper, as the sulphide, according to the procedure described by HEWITT and BOLLE-JONES (1952) whose method for the preparation of molybdenum-free ferric citrate was also followed with but slight modification. This modification consisted of extracting the ether extracted ferric chloride with a solution of dithiol (1, 2-dimercapto-4-methyl-benzene) in amyl acetate before combining it with molybdenum-free citric acid to form ferric citrate. The salts of the micronutrient elements were all recrystallised twice from glass distilled water.

Eighteen seeds, each weighing more than five grams, were sown in each pyrex vessel on 4 January 1955. After a preliminary thinning out, complete plants were removed for analysis in April 1955, May, August, October, December 1955, May 1956. At the earlier samplings two or more seedlings were removed from each vessel at each sampling until at the final, May, sampling one seedling only remained. Seedlings taken from vessels within a treatment replicate were bulked together and apportioned into laminae, stems and roots each of which were separately analysed according to methods previously described (BOLLE-JONES 1954, BOLLE-JONES, MALLIKARJUNESWARA & RATNASINGAM 1957).

RESULTS

Visual and Growth Effects

Molybdenum deficient seedlings which were not supplied with ammonium ions (that is, the –Mo(N) plants) were smaller and paler than those given molybdenum. Unfortunately, iron deficiency symptoms in the upper part of the plants interfered with the true interpretation of the molybdenum deficiency effects. The nearest approach to a consistent effect was the tip or even marginal scorch which occurred in the midstem leaves. The molybdenum deficient seedlings which received a supply of ammonium ions (that is, the –Mo(Am) plants) were smaller and slightly less vigorous than those given molybdenum but did not show any distinctive symptoms which could be attributed to molybdenum deficiency.

Figure 1 illustrates the order of the differences obtained between seedlings grown in different treatments in respect of height, girth and dry weight per plant. The beneficial effect of added molybdenum on these values illustrated the importance of molybdenum in the nutrition of hevea. The height, girth and dry weight per plant values tended to be smaller for the molybdenum deficient plants which were not supplied with ammonium salts. A deficiency of molybdenum retarded growth more severely when nitrogen was supplied entirely as nitrate salts.

Chemical Composition of Plant

The molybdenum content per plant increased markedly with time for the complete nutrient plants; during the first part of the experiment, the molybdenum content per plant was greater in the plants not given ammonium ions (*Figure 1*). However towards the end of the experiment, when iron deficiency effects intervened, the molybdenum content of the Compt.(N) plants was less than for the Compt.(Am) plants.

Statistical examination of the variation of the concentration of molybdenum in laminae taken from different storey positions showed that there was no significant

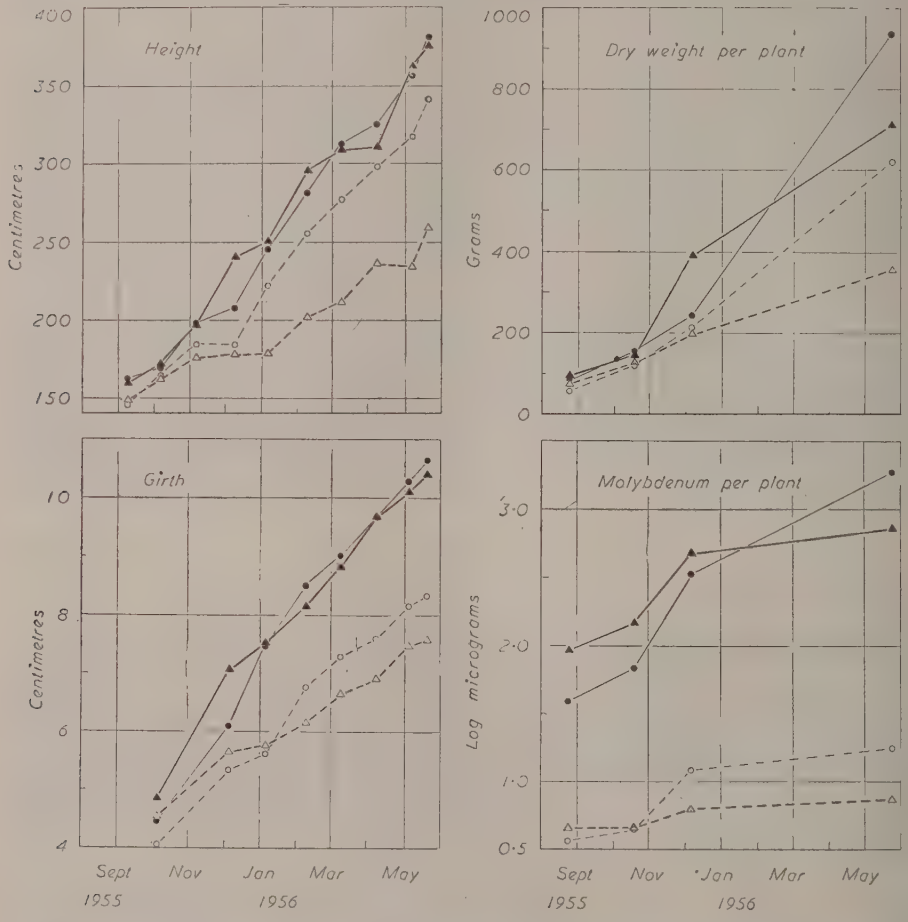

Figure 1. Height, stem girth, dry weight per plant and weight of molybdenum contained in each plant. Girth measured 7 cm above sand level; weight of molybdenum presented as logarithm of microgram value.
Compt.(N)▲ –Mo(N)△ Compt.(Am)● –Mo(Am)○

gradient, according to storey position, in any of the samplings examined. Neither was there established a significant interaction between treatment and storey position for any of the components which were determined by analysis. Consequently the results of the analyses of the laminae are presented as means without reference to storey positions. As the trends for each individual sampling were similar, it is unnecessary to present each separately. The mean values which are presented in Table 1, in respect of stems and roots as well as of laminae, are based on the analyses of plants sampled in August 1955, October, December 1955, May 1956.

The concentrations of molybdenum in the laminae and stems of the Compt.(N) plants were higher than for the Compt.(Am) plants; there were no marked differences between the concentrations found in the corresponding molybdenum deficient laminae and stems (Table 1). For both laminae and roots the absence of molybdenum from the nutrient significantly reduced the concentration of molybdenum in the tissue. It was noteworthy that, for ammonium-supplied plants, a deficiency of molybdenum did not reduce, significantly, the concentration of molybdenum in the stems. It appeared that severe visual symptoms of molybdenum deficiency would not be consistently obtained until the concentration of molybdenum in the dried laminae was well below 0.07 p.p.m., probably at a level of about 0.01 p.p.m. Unexpectedly, it was found that the concentration of molybdenum was much higher in the roots of the Compt.(Am) than in the roots of the Compt.(N) plants (Table 1). Thus the Compt.(Am) plants tended to accumulate a much higher concentration of molybdenum in their roots (4.66 p.p.m.) than in the laminae (0.29 p.p.m.) whereas the Compt.(N) plants, which were not given ammonium ions, possessed a roughly similar concentration in both roots (2.00 p.p.m.) and laminae (1.64 p.p.m.).

A lack of molybdenum depressed the concentration of chlorophyll in the laminae of the nitrate plants but did not affect the concentration found for the ammonium supplied plants (Table 1).

Variation in molybdenum status did not produce any significant effect on the concentrations of rubber found in the petioles, stems or roots (Table 1). However, there were indications that for the roots a deficiency of molybdenum tended to increase the concentration of rubber in the tissues.

The concentrations of total nitrogen in the laminae, stems and roots were greater in the plants supplied with ammonium ions than for those given nitrate nitrogen only (Table 1). Molybdenum deficiency did not effect the concentration of nitrogen in the roots but usually depressed that found in the laminae and stems.

Significant differences in phosphorus and calcium concentrations due to molybdenum level were only established in respect of the roots of the nitrate-fed plants; a deficiency of molybdenum reduced the concentrations of phosphorus and calcium (Table 1). Molybdenum level did not significantly affect the concentrations of magnesium, potassium or manganese in the tissues analysed. A lack of molybdenum generally reduced the iron concentration in the tissues, but only in respect of the laminae and roots of plants which were not fed with ammonium ions was this effect significant (Table 1).

DISCUSSION AND CONCLUSIONS

Molybdenum was thus shown to be an element required for the wellbeing of the rubber plant; its absence was better reflected, in terms of growth and other effects, if ammonium ions were omitted from the nutrients. A molybdenum deficient nutrient

Table 1. Chemical Composition of Laminae, Stems, and Roots

Expressed as % or p.p.m. of oven dried materials

Treatment	Mo p.p.m.	N %	P %	Mg %	K %	Ca %	Fe p.p.m.	Mn p.p.m.	Rubber* %	Chlorophyll %
					Laminae					
Complete (N)	1.64	4.26	0.30	0.28	1.83	0.76	124	42	1.29	0.83
-Mo (N)	0.07	3.86	0.31	0.30	2.04	0.79	110	44	1.38	0.75
Complete (Am)	0.29	4.82	0.50	0.24	1.25	0.49	144	48	1.12	1.14
-Mo (Am)	0.08	4.52	0.48	0.26	1.38	0.50	137	39	1.20	1.12
Min. 5% sig. diff.	0.200	0.151	0.041	0.045	0.223	0.098	11.6	9.7	0.133	0.060
					Stems					
Complete (N)	0.54	0.97	0.23	0.27	1.26	0.62	29	20	0.22	
-Mo (N)	0.03	0.89	0.24	0.27	1.24	0.62	26	22	0.28	
Complete (Am)	0.11	1.18	0.30	0.20	0.86	0.31	50	24	0.23	
-Mo (Am)	0.03	0.98	0.32	0.18	0.81	0.32	26	21	0.24	
Min. 5% sig. diff.	0.294	0.145	0.066	0.065	0.233	0.092	39.2	8.1	0.079	
					Roots					
Complete (N)	2.00	1.18	0.46	0.32	1.22	0.75	886	102	0.30	
-Mo (N)	0.02	1.15	0.34	0.29	1.02	0.51	402	71	0.36	
Complete (Am)	4.66	1.55	0.46	0.20	1.16	0.24	1374	16	0.31	
-Mo (Am)	0.04	1.55	0.44	0.20	1.18	0.22	1152	18	0.38	
Min. 5% sig. diff.	0.102	0.249	0.032	0.050	0.341	0.098	284.6	43.1	0.079	

*Rubber in laminae not given, but concentration in petioles.
N : plants received nitrate but no ammonium nitrogen.
Am : plants received both ammonium and nitrate nitrogen.

in which all nitrogen was given as nitrate grew plants whose concentrations of chlorophyll, nitrogen and iron in the laminae and of phosphorus, calcium and iron in the roots were each significantly reduced. The effects of molybdenum status on height, girth and dry weight per plant also illustrated the undoubted importance of molybdenum in the nutrition of hevea (*Figure 1*). It was unfortunate that the experiment did not lead to a more definite characterisation of the foliar symptoms of molybdenum deficiency other than the tip and marginal scorch.

To confirm and possibly amplify the findings of the experiment described above, another was started about one and a half years later and is still in progress. This later experiment differed from its predecessor in that the iron was supplied not as the citrate but as the ferric form of disodium ethylene-diamine-tetra-acetate, with the object of eliminating iron deficiency effects. Regrettably, the use of this chelated form of iron supply, at a third of the concentration given as citrate in the previous experiment, induced zinc deficiency effects. It was necessary to restore the application of ferric citrate at the rate of 1.0 milligram equivalents per litre in order to eliminate iron deficiency and other complicating effects from the plants not given ammonium ions. Seedlings were removed nine months after the initiation of this second experiment for analysis. The results of this analysis confirmed broadly the findings of the previous experiment with the additional information that the presence of ammonium ions in the nutrient, although decreasing the requirement for molybdenum, greatly increased the concentration of sulphur in the tissues. The concentration of molybdenum in the laminae of the molybdenum deficient nitrate fed plants became reduced to the order of 0.03 p.p.m., in terms of dry tissue, but indubitable symptoms of molybdenum deficiency were not noted nor recognised. The concentration of molybdenum within the tissues was lower for complete nutrient plants supplied with ammonium ions except in the case of roots where the nitrate-fed plants possessed the lower concentration. The dry weight per plant of the nitrate-fed seedlings was slightly greater than that of the seedlings supplied with ammonium ions; similar differences existed in regard to height and girth.

Molybdenum Status as Affected by Calcium and Sulphate Levels

EXPERIMENTAL

The relative importance of molybdenum, sulphur and calcium was evaluated in a factorial experiment in which Tjirandji 1 seedlings were grown in sand at each of the 18 combinations of three levels of molybdenum, three levels of sulphate and two of calcium. These levels represented the following concentrations (in milligram equivalents per litre).

Mo_1 ... 0.00001 MoO_4^{--}	S_1 ... 0.3 SO_4^{--}	Ca_1 ... 0.5 Ca^{++}
Mo_2 ... 0.001 MoO_4^{--}	S_2 ... 1.8 SO_4^{--}	Ca_2 ... 5.0 Ca^{++}
Mo_3 ... 0.1 MoO_4^{--}	S_3 ... 10.8 SO_4^{--}	

Each treatment was carried out in duplicate, thus giving a total of 36 plots; each plot contained 3 pots or vessels. Five-gallon clay pots, internally painted with bitumen, were used for the Mo_2 and Mo_3 treatments but the Mo_1 plants were grown in six-

gallon pyrex vessels. The sand (particle size 0.5 to 1.2 mm) contained in the Mo_2 and Mo_3 pots was purified by a cold acid leaching (5% HCl, v/v) for a three day period. The Mo_1 pyrex vessels were filled with sand which was purified by a seven hour washing with a hot (100°C) hydrochloric (14% w/v) oxalic (1% w/v) acid mixture.

The nutrient solutions were prepared and purified in the same manner as for the experiment earlier described in this paper. For each treatment the following ionic concentrations were maintained (in milligram equivalents per litre): NH_4^+ 3.0, NO_3^- 5.0, PO_4^{---} 3.0, Mg^{++} 2.5, K^+ 3.0, Fe^{+++} 1.0, Mn^{++} 0.02, Cu^{++} 0.002, Zn^{++} 0.002, BO_3^{---} 0.033, Al^{+++} 0.001, Ni^{++} 0.0002, Co^{++} 0.0002, Ga^{+++} 0.0002. Variations in the sodium and chloride ionic concentrations with increased sulphate level were unavoidable but were kept to a minimum; these variations were not thought to be of great importance as a deficiency or toxicity level of either sodium or chloride was avoided.

Eighteen seeds, each weighing more than four grams, were sown in each pot or vessel on 23 April 1955. Plants were removed in August 1955, October 1955, January 1956, April, September 1956, January 1957; all plants taken from any one treatment replicate were bulked together before analysis. The chlorophyll, nitrogen, sulphur phosphorus, magnesium, potassium, calcium, manganese and molybdenum concentrations of the laminae, according to storey position, were determined. The concentration of rubber in the petioles was also determined as well as the total dry weight of each plant. Methods of analysis employed have been previously indicated.

It is unnecessary to present all the results obtained from this experiment here; instead, the values obtained for the September 1956 sampling are considered in some detail as they were representative of the main trends observed. Data obtained from other months are also discussed in the presentation of the results.

RESULTS

Visual and Growth Effects

Throughout the duration of the experiment, the Mo_1 plants possessed fewer leaflets and a smaller height, girth and total dry weight than the Mo_2 plants (*Figure 2*, Table 2). These significant effects of molybdenum were very pronounced in respect of the girth and dry weight values. The Mo_3 plants were consistently shorter than the Mo_2, but in respect of leaflet number, girth and dry weight there appeared little difference between the effects of the two levels. Calcium level did not significantly affect any of these measurements of growth but sulphate application was responsible for beneficial linear effects which were significant (Table 2). Thus the beneficial effect of molybdenum level on growth was almost entirely accounted for by the difference between the Mo_1 and Mo_2 levels whereas the increment in growth measurement values obtained by increasing sulphate from S_2 to S_3 was almost as great as that obtained for the difference between the S_1 and S_2 values (Table 2).

Visual symptoms due to the applied nutrient levels were slow to appear. A dull brownish green appearance of some of the midstem and lower leaves became apparent in the Mo_3S_1 and S_2 plants about a year after sowing. The brownish appearance was caused by a purple tinting of the leaves, which was well marked on the underside of the lamina. These symptoms were ascribed to molybdenum toxicity effects and were noted only in the Mo_3 plants. The symptoms persisted until September 1956 or later

TABLE 2. HEIGHT, STEM GIRTH AND DRY WEIGHT OF SEEDLINGS RECORDED IN JANUARY 1957

Girth measured 7 cm above sand level

Level	Mo_1	Mo_2	Mo_3	S.e.	Ca_1	Ca_2	S.e.	Mean	S.e.
				Height (cm)					
S_1	284	395	361		361	332		347	
S_2	306	419	408	±12.1	391	364	±9.9	378	±7.0
S_3	358	440	438		408	416		412	
Ca_1	321	426	413	±9.9				387	±5.7
Ca_2	311	410	392					371	
Mean	316	418	402	±7.0	387	371	±5.7		
				Girth (cm)					
S_1	9.4	13.0	13.1		12.4	11.2		11.8	
S_2	9.9	13.7	14.0	±0.38	12.8	12.3	±0.31	12.5	±0.22
S_3	11.3	14.6	15.4		13.6	14.0		13.8	
Ca_1	10.4	14.2	14.2	±0.31				12.9	±0.18
Ca_2	10.0	13.4	14.1					12.5	
Mean	10.2	13.8	14.2	±0.22	12.9	12.5	±0.18		
				Dry Weight per Plant (gm)					
S_1	559	1132	1120		1039	835		937	
S_2	688	1451	1339	±135	1204	1115	±110	1159	±78
S_3	881	1497	1633		1334	1340		1337	
Ca_1	747	1413	1418	±110				1192	±64
Ca_2	672	1308	1310					1097	
Mean	710	1360	1364	±78	1192	1097	±64		

but disappeared by the end of the year. It seemed, as was also apparent in the growth measurement values, that during the earlier part of the experiment the Mo_3 level exercised a mild toxic effect on growth but that later even this high level benefited growth, particularly when recorded as girth (Table 2).

In June 1956 the first indications of leaf symptoms believed to be those of molybdenum deficiency, were recorded. The tips and margins became scorched without any preliminary chlorosis of the leaf tissues. The scorched area which was thin, paper-like and fawn in colour could extend all around the lamina margin or it could be confined to the tip region with its base line running transversely across the lamina (*Figures 3* and *4*). The scorch was not necessarily preceded by a paling of the lamina and could occur in quite dark green leaflets. The location of the symptom-bearing leaves was difficult to classify. The leaflets were always fully expanded and the symptom usually occurred in the second or third storeys away from the top of the plant (*Figure 3*) but in many instances they extended even into the top storey leaflets. The symptoms were more frequent and more severe at the Ca_2 level and occurred in all the Mo_1 plants; they were first noted in the Mo_1S_3 treatments and took their most severe form in plants of those treatments.

Mild symptoms of sulphur deficiency were noted in the topmost leaves of the $Mo_3S_1Ca_1$ and $Mo_3S_1Ca_2$ plants in January 1957. They were not severe and did not appear in all plants of the affected treatment.

Chemical Composition Of Laminae

The distribution of molybdenum, in terms of concentration, between storeys seemed to vary according to the level of molybdenum applied in the nutrient (Table 3). The differences between the molybdenum concentrations of the top and second storey laminae of the Mo_1 plants were not significant but for most samplings the second storey laminae contained the lower concentration. The molybdenum concentration values for the Mo_1 plants were of the order of 0.05 to 0.20 p.p.m. of molybdenum in oven dried second storey laminae even in plants showing symptoms suspected to be those of molybdenum deficiency (Table 3); these values were higher than anticipated. For the Mo_2 plants there was every indication that the second storey laminae contained more molybdenum than those of the top storey. This effect was not significant if only the September values were considered but became so if the values for September and January were combined before statistical analysis (Table 3). The laminae of the lower storeys of Mo_3 plants contained much more molybdenum than those of the upper storeys and this effect was significant when data from the September and January samplings were combined prior to analysis (Table 3).

It was necessary for the statistical evaluation of the effect of treatment on molybdenum concentration to examine separately the values for each molybdenum level as the range covered would otherwise tend to obscure any statistically significant effect. Thus the second storey laminae of the Mo_3 plants sometimes contained as much as 1,000 p.p.m. of molybdenum whereas those of the Mo_1 plants contained as little as 0.05 p.p.m. Nevertheless certain consistent effects were detectable. Increased sulphate level consistently reduced the concentration of molybdenum in the laminae, but increased calcium level produced no effect (Table 3). The rubber plant showed itself able to accumulate large concentrations of molybdenum in the older laminae especially at the Mo_3S_1 levels, but accompanied by relatively mild visual indication of any toxicity effect.

One of the well known effects of molybdenum deficiency is the accumulation of nitrate in deficient laminae. To obtain further evidence that the symptoms observed in the Mo_1 plants were those of molybdenum deficiency, nitrate nitrogen was determined in the top storey laminae sampled in January 1957. The result (Table 4) clearly confirmed that nitrate nitrogen accumulated in the laminae of the Mo_1 plants and that a fivefold reduction in nitrate concentration occurred when the molybdenum level was increased from Mo_1 to Mo_2.

The remaining data discussed below were obtained from plants sampled in September 1956 and are presented as means of the top and second storey values, as examination of the individual samplings showed that the trends were similar for both storeys. The concentration of rubber in the petioles diminished, significantly, as the plant's molybdenum status improved (Table 4); the concentration of chlorophyll within the lamina was not affected by any of the applied treatments and is not given here.

Increased supplies of molybdenum significantly decreased the concentration of nitrogen, sulphur, phosphorus, magnesium and potassium in the laminae but increased that of manganese (Tables 5 and 6). Increased sulphate level increased the concentration of potassium in the laminae but decreased those of calcium and manganese (Table 6). The principal effect of increased calcium level, apart from increasing the concentration of calcium in the laminae, was to decrease that of magnesium (Table 6). Negative interactions between molybdenum and sulphate levels which affected the concentrations of sulphur, phosphorus and potassium in the laminae were also noted.

TABLE 3. CONCENTRATION OF MOLYBDENUM IN LAMINAE OF DIFFERENT STOREYS

Values given as p.p.m. Mo in oven dried laminae

Mo_1 plants, September 1956 sampling.

Treatment	Storey		Min. 5% sig. diff.
	top	second	
S_1 Ca_1	0.29	0.12	Between 2 treatment values of same storey 0.26
S_2 Ca_1	0.28	0.16	
S_3 Ca_1	0.10	0.16	
S_1 Ca_2	0.10	0.10	Between 2 different storey values of same treatment 0.23
S_2 Ca_2	0.24	0.06	
S_3 Ca_2	0.16	0.06	
Mean	0.20	0.11	Between overall mean storey values 0.14

Mo_2 plants, September 1956 and January 1957 samplings.

Treatment	Storey		Min. 5% sig. diff.
	top	second	
S_1 Ca_1	562	1011	Between 2 treatment values of same storey 496
S_2 Ca_1	168	410	
S_3 Ca_1	66	138	
S_1 Ca_2	527	1043	Between 2 different storey values of same treatment 374
S_2 Ca_2	201	508	
S_3 Ca_2	54	142	
Mean	263	542	Between overall mean storey values 112

Mo_3 plants, September 1956 and January 1957 samplings.

Treatment	Storey		Min. 5% sig. diff.
	top	second	
S_1 Ca_1	8.22	13.80	Between 2 treatment values of same storey 2.33
S_2 Ca_1	3.17	4.24	
S_3 Ca_1	1.74	2.34	
S_1 Ca_2	8.69	11.81	Between 2 different storey values of same treatment 2.46
S_2 Ca_2	3.90	5.56	
S_3 Ca_2	1.68	1.95	
Mean	4.56	6.62	Between overall mean storey values 1.30

TABLE 4. THE CONCENTRATION OF NITRATE NITROGEN IN TOP STOREY LAMINAE, SAMPLED IN JANUARY 1957, AND OF RUBBER IN TOP AND SECOND STOREY PETIOLES, SAMPLED IN SEPTEMBER 1956

Expressed as p.p.m. or % of oven dried materials

p.p.m. Nitrate – N

Level	Mo_1	Mo_2	Mo_3	S.e	Ca_1	Ca_2	S.e.	Mean	S.e.
S_1	584	77	80		299	195		247	
S_2	406	92	136	±94.6	235	188	±77.2	211	±54.6
S_3	638	128	137		286	316		301	
Ca_1	602	80	138	±77.2				273	±44.6
Ca_2	484	118	97					233	
Mean	543	99	117	±54.6	273	233	±44.6		

Rubber %

Level	Mo_1	Mo_2	Mo_3	S.e.	Ca_1	Ca_2	S.e.	Mean	S.e.
S_1	1.45	1.08	1.12		1.20	1.23		1.22	
S_2	1.68	1.05	1.05	±0.070	1.36	1.16	±0.057	1.26	±0.041
S_3	1.33	1.23	1.18		1.29	1.20		1.25	
Ca_1	1.57	1.13	1.15	±0.057				1.28	±0.033
Ca_2	1.40	1.11	1.08					1.20	
Mean	1.49	1.12	1.12	±0.041	1.28	1.20	±0.033		

GENERAL DISCUSSION

These investigations clearly indicate that a deficiency of molybdenum will markedly reduce the height, girth and dry weight of the rubber plant (*Figures 1* and *2*, Table 2). These effects appeared even when the plants were given a large proportion of their nitrogen supply as ammonium ions, suggesting that molybdenum was probably essential for some reaction other than one involved directly in nitrate reduction. Symptoms of molybdenum deficiency are usually recorded in plants supplied with nitrogen as nitrate and it is much more difficult to produce symptoms in the same plants when given part or all of their nitrogen as ammonium; it has been reported that a deficiency of molybdenum in tomato was not accompanied by symptoms when a non-nitrate source of nitrogen was supplied (HEWITT & MCCREADY 1956). However, other reports (VANSELOW & DATTA 1949) show that symptoms of molybdenum deficiency may be produced even in plants supplied with ammonium nitrogen.

The present results indicate that the rubber plant may also produce symptoms (*Figures 3* and *4*) when supplied with a molybdenum deficient nutrient (Mo_1) containing ammonium nitrogen. It was unfortunate that the interpretation of similar symptoms in plants not given ammonium nitrogen was rendered impracticable due to the occurrence of complicating iron deficiency effects. An anomalous factor regarding the present results was the occurrence of the supposed molybdenum deficiency symptoms in plants grown at the Mo_1 level (of the second experiment reported here) whereas no symptoms were observed in the leaves of the –Mo(Am) plants (of the first experiment) even though the concentrations of molybdenum in the laminae were

TABLE 5. CONCENTRATION OF NITROGEN, SULPHUR AND PHOSPHORUS IN TOP AND SECOND STOREY LAMINAE, SEPTEMBER 1956 SAMPLING

Expressed as % of oven dried materials

Level	Mo_1	Mo_2	Mo_3	S.e.	Ca_1	Ca_2	S.e.	Mean	S.e.
			Nitrogen %						
S_1	4.17	3.73	3.85		3.95	3.88		3.92	
S_2	4.17	3.78	3.71	±0.094	3.88	3.90	±0.77	3.89	±0.054
S_3	4.17	3.90	3.79		3.97	3.94		3.95	
Ca_1	4.16	3.77	3.87	±0.077				3.93	±0.044
Ca_2	4.17	3.84	3.70					390	
Mean	4.17	3.81	3.78	±0.054	3.93	3.90	±0.044		
			Sulphur %						
S_1	0.18	0.16	0.15		0.16	0.16		0.16	
S_2	0.25	0.21	0.21	±0.019	0.21	0.24	±0.016	0.22	±0.011
S_3	0.43	0.22	0.23		0.28	0.30		0.29	
Ca_1	0.28	0.19	0.18	±0.016				0.22	±0.009
Ca_2	0.29	0.20	0.21					0.23	
Mean	0.28	0.19	0.20	±0.011	0.22	0.23	±0.009		
			Phosphorus %						
S_1	0.46	0.30	0.27		0.37	0.32		0.34	
S_2	0.41	0.25	0.26	±0.022	0.31	0.30	±0.018	0.31	±0.013
S_3	0.54	0.26	0.25		0.37	0.33		0.35	
Ca_1	0.50	0.28	0.27	±0.018				0.35	±0.010
Ca_2	0.45	0.26	0.25					0.32	
Mean	0.47	0.27	0.26	±0.013	0.35	0.32	±0.010		

roughly of the same order (Tables 1 & 3). Differences in cultural conditions might account for this anomaly. Thus the Mo_1 plants were grown in the open air, exposed to full sunlight, whereas the –Mo(Am) plants were grown under light shade in the glasshouse; shading might well have tended to reduce the threshold deficiency concentration level for the appearance of molybdenum deficiency symptoms in the glasshouse grown plants.

The occurence of marginal leaf scorch was clearly correlated with a low molybdenum status as it was largely confined to the Mo_1 plants and increased in severity as the sulphate level increased. These leaves also accumulated nitrate nitrogen (Table 4) and this is one of the effects of molybdenum deficiency (ANDERSON 1956.) It is believed that these symptoms were a true manifestation of molybdenum deficiency but complete acceptance of this conclusion must be reserved owing to the rather high molybdenum concentration values recorded in the laminae of some of the plants which showed symptoms. Under these circumstances it is difficult to predict the concentration value for molybdenum in laminae at which symptoms of molybdenum deficiency would appear. The first experiment reported here would seem to indicate a threshold value of about 0.01 p.p.m. of molybdenum in dry laminae, but the second experiment indicates a value which would be closer to 0.10 p.p.m. However, there is sufficient evidence on which to base a method of dealing with leaf samples obtained in

TABLE 6. CONCENTRATION OF MAGNESIUM, POTASSIUM, CALCIUM AND MANGANESE IN TOP AND SECOND STOREY LAMINAE, SEPTEMBER 1956 SAMPLING

Expressed as % of oven dried materials

Level	Mo_1	Mo_2	Mo_3	S.e.	Ca_1	Ca_2	S.e.	Mean	S.e.
				Magnesium %					
S_1	0.32	0.24	0.23		0.29	0.25		0.27	
S_2	0.30	0.26	0.24	±0.017	0.30	0.24	±0.014	0.27	±0.010
S_3	0.34	0.22	0.23		0.30	0.23		0.26	
Ca_1	0.34	0.27	0.27	±0.014				0.29	±0.008
Ca_2	0.30	0.21	0.20					0.24	
Mean	0.32	0.24	0.24	±0.010	0.29	0.24	±0.008		
				Potassium %					
S_1	1.23	1.02	1.19		1.13	1.16		1.14	
S_2	1.10	1.00	0.97	±0.046	1.01	1.04	±0.038	1.02	±0.027
S_3	1.48	1.05	1.10		1.21	1.21		1.21	
Ca_1	1.29	1.02	1.04	±0.038				1.12	±0.022
Ca_2	1.25	1.02	1.14					1.14	
Mean	1.27	1.02	1.09	±0.027	1.12	1.14	±0.022		
				Calcium %					
S_1	0.49	0.44	0.47		0.26	0.67		0.47	
S_2	0.45	0.51	0.50	±0.027	0.26	0.72	±0.022	0.48	±0.015
S_3	0.31	0.46	0.39		0.24	0.54		0.39	
Ca_1	0.20	0.28	0.28	±0.022				0.25	±0.012
Ca_2	0.63	0.66	0.63					0.64	
Mean	0.42	0.47	0.45	±0.015	0.25	0.64	±0.012		
				Manganese p.p.m.					
S_1	57	76	74		69	69		69	
S_2	55	85	82	±5.3	67	81	±4.3	74	±3.0
S_3	36	75	66		62	56		59	
Ca_1	47	76	74	±4.3				66	±2.5
Ca_2	51	81	74					68	
Mean	49	79	74	±3.0	66	68	±2.5		

the course of advisory or extension work. Thus, any leaf sample which shows well defined marginal scorch, an absence of severe chlorosis and which possesses a concentration of molybdenum within the laminae of the order of 0.05 p.p.m. may be suspected of being molybdenum deficient. In such instances field application of molybdenum is warranted. Once the field occurrence of molybdenum deficiency has been established with confidence and growth responses to molybdenum application obtained it should become relatively simple to establish the threshold concentration of molybdenum in laminae.

Figure 2. $Mo_2S_1Ca_2$ left, $Mo_1S_1Ca_2$ right. Note retardative effect of molybdenum deficiency on growth and occurrence of scorch in lowermost leaves of Mo_1 plant. Mo_2 seedling about 11 feet tall. September 1956.

It was beyond doubt that the tissues of the shoots of rubber plants supplied with a complete nutrient which contained ammonium nitrogen contained a smaller concentration of molybdenum than plants which did not receive ammonium nitrogen (Table 1). However, this did not necessarily imply that the absolute requirement for molybdenum was smaller as the roots of the Compt.(Am) plants contained a higher concentration of molybdenum than those of the Compt.(N) plants. For the earlier part of the experiment it was true that the weight of molybdenum contained per plant was higher in the Compt.(N) plants but this difference diminished with time and became reversed as the Compt.(Am) plants outstripped their nitrate fed counterparts (*Figure 1*). Under conditions of molybdenum deficiency the concentration of molybdenum in the laminae of both ammonium and nitrate fed plants fell to about the same level (Table 1) but the plants not given ammonium nitrogen became inferior in terms of height, girth and dry weight (*Figure 1*). Some effects on chemical composition also showed that the effect of deficient molybdenum supply was more acutely felt in the absence of a supply of ammonium nitrogen. Thus, as also recorded by Hewitt and McCready (1956), a deficiency of molybdenum reduced the concentration of chlorophyll in the laminae when nitrogen was supplied as nitrate but not if given ammonium nitrogen (Table 1). The concentrations of iron in the laminae and of phosphorus, calcium and iron in the roots were significantly reduced in the –Mo(N) plants but not in the –Mo(Am) plants (Table 1).

Increased sulphate level diminished the concentration of molybdenum in the laminae (Table 3) and fully confirmed the initial finding of Stout et alii (1951) who showed that sulphate application depressed molybdenum uptake. Heavy sulphate application, especially under acid soil conditions, may therefore be conducive to the production of molybdenum deficiency effects. The present work shows that these effects need not necessarily be represented as visual symptoms but may take the form of reduced growth. Accordingly it is credible that under Malayan conditions there may be instances of retarded growth which are caused by a lack of molybdenum. This is a possibility which should be investigated under field conditions.

Increased calcium supply seemed to increase the severity of the supposed molybdenum deficiency symptoms and there were indications that the concentration of molybdenum

in the Mo_1Ca_2 laminae was less than for the Mo_1Ca_1 plants (Table 3). However, this difference was far short of being significant and was certainly not applicable to the Mo_2 and Mo_3 plants. It did not seem that calcium had a profound effect on the molybdenum nutrition of the rubber plant although its effect may well have been greater if ammonium ions had not been supplied in the nutrient. The beneficial effect of lime on molybdenum uptake from soils must be attributed to its effect (presumably one of pH) on the availability of molybdenum in the soil and not to any specific effect of the calcium ions. It has also been reported (WARRINGTON 1950) that, in solution culture, the amount of applied calcium did not affect the plant's response to molybdenum.

It is usually supposed that there is some form of antagonism between molybdenum and manganese (MULDER 1954) so that applied manganese would depress molybdenum uptake. The present work shows that, unlike most of the other mineral nutrients determined, the concentration of manganese in the laminae increased as the molybdenum supply increased (Table 6). However, this effect may only be of significance when ammonium nitrogen is supplied in the nutrient (Table 1).

Figure 3. Leaves of second storey of $Mo_1S_1Ca_2$ plant. Note marginal scorch believed due to molybdenum deficiency. January 1957.

Figure 4. Leaflets of $Mo_1S_3Ca_2$ plant. Note severe marginal and tip scorch and relatively slight mottling. September 1956.

SUMMARY

Rubber seedlings, in sand culture, were supplied with molybdenum deficient nutrients in which the nitrogen was supplied as nitrate or as a mixture of nitrate and ammonium ions. Other seedlings were grown at different levels of molybdenum, sulphate and calcium.

Seedlings deprived of molybdenum or given a very low level of supply were shorter, of smaller girth and dry weight, and developed a marginal or tip scorch in the midstem and upper laminae. Increased sulphate level improved their growth despite the usually lowered molybdenum concentration in the laminae.

Molybdenum deficient plants, given nitrogen as nitrate ions, possessed smaller concentrations of chlorophyll and iron in the laminae and of phosphorus, calcium and iron in the roots, than those given molybdenum. Complete nutrient seedlings supplied with ammonium nitrogen contained a greater concentration of molybdenum in their roots and lower concentrations in their stems and laminae than those given nitrate only.

Although not established beyond doubt, it appeared that under conditions of molybdenum deficiency the topmost storey of laminae contained a higher concentration of molybdenum than the second storey in which the symptoms often first appeared. At normal and high levels of molybdenum supply the older laminae possessed a higher concentration than the younger ones. Mild symptoms of molybdenum toxicity were observed in the older leaves at the highest level of molybdenum supply.

Increased molybdenum supply diminished the concentration of nitrate nitrogen within the laminae as well as the concentrations of sulphur, phosphorus, magnesium and potassium, but increased that of manganese.

Increased sulphate supply diminished the concentration of molybdenum in the laminae; calcium had no significant effect but appeared to accentuate the severity of the symptoms of molybdenum deficiency.

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Soils Division
Rubber Research Institute of Malaya
Kuala Lumpur

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Zinc: Effects on the Growth and Composition of Hevea

E. W. BOLLE-JONES

Zinc deficiency symptoms were produced in hevea seedlings grown in sand. The nature of the symptoms and the effects of the deficiency on growth and composition of the seedlings are described.

ZINC IS ONE OF THE ESSENTIAL MICRONUTRIENT or trace elements required by plants. The importance and essentiality of zinc to the nutritional wellbeing of fruit trees has been well demonstrated (CHILDERS 1954) but a complete lack of information existed on its significance to *Hevea brasiliensis*. Zinc deficiency may be expected to occur in plants grown on alkaline soils, on some sandy soils subjected to heavy cropping or on some heavier soils in which the zinc may be bound by organic compounds. It was not thought likely that zinc deficiency would be a prevalent cause of malnutrition in rubber trees grown on the generally acid soils of Malaya. Nevertheless the readiness with which zinc deficiency effects were produced in the investigation reported here suggested that the rubber tree may possess a relatively high zinc requirement. These results provide information which may be used to prove or disprove the occurrence of zinc deficiency under field conditions in Malaya.

The experiment consisted of growing rubber plants in sand under conditions of zinc deficiency and supplying them with nitrogen either as nitrate or as a mixture of nitrate and ammonium salts. The results, which are presented in brief form, show clearly the well defined effects of zinc deficiency not only on growth and visual appearance but also on chemical composition. It was also noted that zinc deficiency appeared to predispose rubber seedlings to infection by *Odium heveae*; a note on this aspect has been published elsewhere (BOLLE-JONES & HILTON 1956).

EXPERIMENTAL

Selfed seedlings of clone Tjirandji 1 were grown in sand at two levels of zinc supply and at different levels of nitrate and ammonium supply. The four treatments were carried out in duplicate and consisted of the following levels of zinc, nitrate nitrogen and ammonium nitrogen (in milligram equivalents per litre).

Treatment	Level		
	Zn^{++}	NO_3^-	NH_4^+
– Zn (N)	0	8	0
Compt. (N)	0.002 (0.065 p.p.m.)	8	0
– Zn (Am)	0	5	3
Compt. (Am)	0.002 (0.065 p.p.m.)	5	3

The preparation of pyrex vessels and sand and the composition of the basal nutrient, which were as employed in the investigation of molybdenum deficiency effects, have been previously described (Bolle-Jones 1957a). Water obtained by demineralisation of rainwater by passage through ion exchange resin units was used to prepare the nutrient solutions. The nutrient salts were cleansed of zinc by the same methods as used for the removal of copper and which have been described elsewhere (Bolle-Jones 1957b).

Eighteen Tjirandji 1 seeds, each weighing more than five grams, were sown in each pyrex vessel on 4 January 1955. Seedlings, after the initial thinning out, were removed for analysis in the following April, May, July, November 1955, January and February 1956. The plants from each treatment replicate were bulked together and divided into laminae (excluding midribs), stems and petioles, and roots, prior to analysis. The zinc deficient plants developed such severe symptoms that it was necessary to supply them with zinc during the periods 20 July to 4 August (0.23 mg Zn supplied per plant) and 16 September to 29 October (0.83 mg Zn supplied per plant) in order to keep them alive.

RESULTS

Visual and Growth Effects

The height, stem girth and dry weight of each zinc deficient plant was much less than for the corresponding complete nutrient (Compt.) plant (*Figures 1* and *2*). The apparent improvement in height, girth and dry weight values of the – Zn(N) plants which occurred towards the end of the experiment reflected the effect of the added zinc in the July to October period; the recovery of the – Zn(Am) plants was less. The severity of the effects of zinc deficiency on growth was greater in the – Zn(Am) than in the – Zn(N) plants.

Foliar symptoms of zinc deficiency were first apparent in both – Zn(N) and – Zn(Am) seedlings three months after sowing, and were localised in the upper and youngest storeys of leaves (*Figure 3*). The youngest leaflets became incurved towards one another and presented a hook or claw appearance (*Figures 3* and *4*). At this stage chlorotic patches could occur between the veins (*Figure 4*) and the margins of the laminae became wavy and frequently irregular. After passing through this stage the leaflets produced laminae which were much reduced in breadth in proportion to their length (*Figures 5* and *6*). Frequently there occurred a compression of the apical internodes to give a rosette appearance and the narrow green laminae became pronged by yellow areas which spread inwardly and interveinally from the margins (*Figures 5* and *6*). The expanded upper storey leaves of the zinc deficient plants were of much smaller size than those of the complete nutrient plants and also frequently became twisted (*Figure 6*). The interveinal chlorotic areas sometimes became brown and necrotic.

As the symptoms became progressively severe the young tender leaflets failed to expand and withered and died; the youngest leaf initials became yellow or white and the stem growing point died. Other, axillary, shoots or sprouts would be formed in axils of leaves lower down the stem (*Figure 2*) but often times these also turned yellow and withered. Finally new shoots would arise from sand level to make a partial recovery and provide some expanded leaves but later these shoots also died. In consequence of these acute symptoms which appeared more severe in the – Zn(Am) than in the – Zn(N) plants it was necessary to resuscitate the plants with zinc in July as previously indicated.

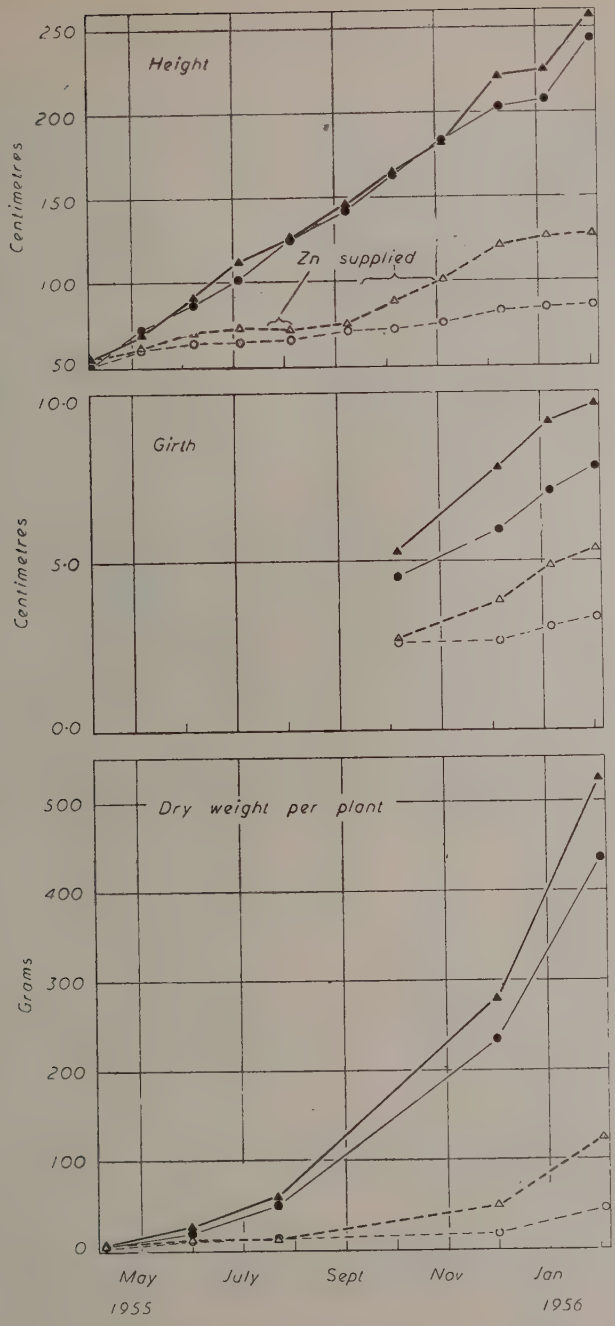

Figure 1. Height, stem girth, and dry weight per plant. Girth measured 7 cm above sand level.

Compt.(N)▲ – Zn(N)△

Compt.(Am)● – Zn(Am)○

A deficiency of zinc predisposed the seedlings to infection by *Oidium heveae* which, in many instances, was found to envelop the stem growing points of the zinc deficient plants (Bolle-Jones & Hilton 1956). *Oidium* attack was partially responsible for some of the distortion observed in zinc deficient laminae and for some of the loss of young leaves. Subsequent experiments have, however, shown that when rubber seedlings were grown in an air-conditioned glasshouse in the absence of *Oidium* that the symptoms described above were characteristic of the deficiency and not secondary features which arose because of the *Oidium* infection.

Chemical Composition of Plant

Examination of the analytical data obtained revealed that treatment effects were broadly similar for each sampling. The results obtained for plants sampled in May 1955 and February 1956 are presented herein. The May sampling was taken before the zinc deficient plants were resuscitated and the February sampling was taken when the plants were again showing severe effects of zinc deficiency even after having received zinc some four months previously.

The zinc content per plant was many times greater in the complete nutrient (Compt.) than in the zinc deficient plants and this was accompanied by similar disparities in terms of dry weight per plant (Table 1, *Figure 1*). However, the ratio Compt./ – Zn was approximately twice as great for the zinc content per plant values as for the dry weight values.

For the experiment described here the concentration of zinc in the laminae was obtained by analysis of the bulked laminae, regardless of storey position. The results so obtained indicated that values of the order of 12 to 15 p.p.m. of zinc were found in laminae which were zinc deficient and that healthy laminae contained 16 to 21 p.p.m. or more zinc (as expressed in terms of oven dried laminae). Therefore, the differences between the concentrations of zinc in the deficient and healthy laminae were not arithmetically great but nevertheless could be significant (Table 1) and were accompanied by visual symptoms of the deficiency. The differences between the concentrations of zinc in the zinc deficient and healthy stems and petioles, and roots, were relatively greater than for the laminae but these differences were not always significant (Table 1). For the roots also, this difference increased with time. It appeared that careful standardisation of stem sampling and analysis might give values of importance in diagnostic work. A concentration of 3 p.p.m. of zinc in (oven dried) stems of young seedlings appeared to be associated with zinc deficiency whereas the healthy seedlings contained three or more times this amount.

The results of the present experiment did not provide information on the variation of the concentration of zinc in laminae according to storey position. A later experiment, in which the basal nutrient solution was of similar composition to the – Zn (Am) solution used here, gave results which are relevant and which are shown in Table 2. These results indicated that the uppermost laminae which showed the most acute deficiency symptoms contained the least zinc and that the concentration increased as the leaves grew older.

The effects of zinc supply on components other than zinc are given in Table I. It was evident that zinc status influenced the composition of stems and petioles rather more than that of laminae and much more than the composition of the roots. Many effects due to a deficiency of zinc were recorded but among the more consistent were the following: the increases in the stem and petiolar concentrations of nitrogen, phosphorus, iron and manganese; the increase in the concentration of nitrogen in the roots; evidence of increases in the concentrations of calcium, iron and manganese in the laminae. The source of nitrogen supplied modified the intensity of these effects but seldom altered their nature.

Figure 2. Compt.(Am) left, –Zn(Am) right. Note stunting of zinc deficient seedlings with signs of previous dieback and defoliation. Eleven months old seedlings. Each scale division equal to three inches.

Figure 6. Narrow straplike twisted leaves taken from upper storey of –Zn(Am) seedling.

Figure 5. Top of six months old –Zn(N) seedling. Note narrowness and twisting of laminae.

Figure 3. Top of three months old zinc deficient seedling. Note hook or claw appearance and reduced size of uppermost leaflets.

Figure 4. Single zinc deficient leaf showing severe hooking and chlorosis of lamina which spread interveinally from margin.

TABLE 1. DRY WEIGHT AND TOTAL ZINC CONTENT PER PLANT AND CHEMICAL COMPOSITION OF OVEN DRIED LAMINAE, STEMS AND PETIOLES, AND ROOTS

May 1955 and February 1956 samplings

Treatment	Dry wt per plant gm	Zn content per plant mg	Dry wt per plant gm	Zn content per plant mg
	May 1955		*February 1956*	
Compt.(N)	27	0.41	526	8.86
– Zn(N)	12	0.09	125	0.62
Compt.(Am)	20	0.28	436	8.24
– Zn(Am)	11	0.06	46	0.30
Min. 5% sig. diff.	11.1	0.292	298.7	7.713

Laminae, May 1955

Treatment	Zn p.p.m.	Rubber %	Chloro-phyll %	N %	P %	Mg %	K %	Ca %	Fe p.p.m.	Mn p.p.m.
Compt.(N)	16.0	—	1.00	3.60	0.30	0.34	1.74	0.78	91	105
– Zn(N)	12.4	—	0.92	3.58	0.34	0.32	1.82	1.19	134	326
Compt.(Am)	18.2	—	1.11	3.88	0.31	0.26	1.24	0.52	117	73
– Zn(Am)	12.0	—	1.05	4.78	0.41	0.40	1.22	0.77	128	157
Min. 5% sig. diff.	3.00	—	0.617	0.667	0.126	0.175	0.872	0.386	25.5	202.5

Laminae, February 1956

Compt.(N)	18.1	0.93	0.83	3.64	0.28	0.33	1.72	0.92	130	61
– Zn(N)	12.8	1.04	1.05	3.73	0.34	0.46	1.79	1.64	152	236
Compt.(Am)	21.3	0.76	1.12	4.59	0.34	0.32	1.11	0.55	124	42
– Zn(Am)	13.6	1.12	0.92	4.64	0.42	0.68	1.14	1.72	172	266
Min. 5% sig. diff.	9.63	0.339	0.549	1.620	0.192	0.101	1.188	0.748	56.7	194.7

Stems and petioles, May 1955

Treatment	Zn p.p.m.	Rubber %	N %	P %	Mg %	K %	Ca %	Fe p.p.m.	Mn p.p.m.
Compt.(N)	13.6	0.60	1.04	0.23	0.44	1.38	0.62	20	52
– Zn(N)	4.2	0.64	2.14	0.44	0.26	1.16	0.66	42	101
Compt.(Am)	10.2	0.68	1.62	0.30	0.26	1.00	0.42	38	48
– Zn(Am)	3.0	0.62	2.62	0.50	0.24	1.00	0.38	41	44
Min. 5% sig. diff.	13.27	0.309	0.139	0.060	0.136	0.751	0.120	17.7	40.1

Stems and petioles, February 1956

Compt.(N)	9.0	0.24	1.12	0.18	0.27	1.30	0.66	21	28
– Zn(N)	2.6	0.42	1.75	0.37	0.22	1.27	0.55	40	60
Compt.(Am)	6.5	0.28	1.22	0.24	0.21	0.88	0.34	26	27
– Zn(Am)	4.0	0.50	3.04	0.56	0.24	1.02	0.50	74	59
Min. 5% sig. diff.	5.25	0.209	0.346	0.118	0.081	0.202	0.311	13.9	25.5

Roots, May 1955

Treatment	Zn p.p.m.	Rubber %	N %	P %	Mg %	K %	Ca %	Fe p.p.m.	Mn p.p.m.
Compt.(N)	14.8	—	1.13	0.38	0.42	1.58	0.30	322	99
– Zn(N)	8.8	—	1.88	0.49	0.30	1.64	0.26	510	71
Compt.(Am)	12.4	—	1.64	0.50	0.20	1.51	0.17	736	26
– Zn(Am)	8.5	—	2.27	0.65	0.26	1.50	0.16	756	25
Min. 5% sig. diff.	6.15	—	0.508	0.221	0.139	0.820	0.154	734	40.0

Roots, February 1956

Compt.(N)	38.9	0.34	1.54	0.42	0.36	1.56	0.93	1720	198
– Zn(N)	9.0	0.31	1.56	0.40	0.23	1.37	0.35	1473	102
Compt.(Am)	37.2	0.48	1.76	0.35	0.24	2.00	0.36	2038	46
– Zn(Am)	9.6	0.32	2.44	0.66	0.21	1.26	0.28	1812	44
Min. 5% sig. diff.	30.89	0.112	0.539	0.108	0.286	0.859	0.484	1826	42.9

TABLE 2. CONCENTRATION OF ZINC IN LAMINAE OF DIFFERENT STOREYS

Each value is the mean of two replicate sample determinations, given as p.p.m. Zn in oven dried material.

Treatment	Storey	3 months old seedlings ordinary glasshouse	5 months old seedlings ordinary glasshouse	5 months old seedlings air-conditioned glasshouse	5 months old seedlings open air
Compt. (NH_4)	Top	24	18	26	20
	2nd	21	20	38	22
	3rd	28	34	44	42
	4th	—	37	51	28
– Zn (NH_4)	Top	16	18	15	—
	2nd	18	17	18	—
	3rd	24	19	22	23
	4th	—	21	31	35

DISCUSSION

It was relatively easy to produce symptoms of zinc deficiency in the rubber seedling. These symptoms were well defined and were quite unlike those reported by HEWITT and BULL (1956) and described as suspected zinc deficiency. Well marked differences in growth and composition accompanied the appearance of symptoms (*Figures 1* and 2 and Table 1); moreover, the symptoms appeared first in the youngest leaves which analysis showed to contain less zinc than those lower down the stem (Table 2). Proof, that the observed symptoms really were those of zinc deficiency, was furnished by the recovery of plants which were resuscitated with zinc during the July to October period.

The concentration of zinc in the laminae yielded some measure of the zinc status of the plant but the concentration in the stem might prove to be of greater value in diagnostic work as the disparity between the concentrations of deficient and healthy tissues was much greater for stems than for laminae (Table 1). The stems and petioles also, in several respects, seemed to reflect zinc status more sensitively than the laminae; for example, the highly significant increases in nitrogen and phosphorus under zinc deficient conditions. Such increases were also occasionally shown in the laminae but were not significant (Table 1).

SUMMARY

Symptoms of zinc deficiency were readily produced in rubber seedlings grown in purified sand when supplied with nutrients cleansed of zinc; zinc deficient leaflets were characterised by a hook or claw appearance in the tender stage and later, when expanded, by a reduction in width of the lamina in proportion to its length.

Seedlings deprived of zinc were shorter, of smaller girth and dry weight, and contained much less zinc per plant than seedlings given zinc.

Analysis of stem and petiolar tissues might, with careful standardisation of sampling procedures, provide a better index of zinc status than analysis of the laminae.

A deficiency of zinc was accompanied by increased concentrations of nitrogen and phosphorus in the stems and petioles and of nitrogen in the roots; evidence of accumulation of iron and manganese in all the shoot tissues was also obtained.

Soils Division
Rubber Research Institute of Malaya
Kuala Lumpur

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Nitrogen Fixation by Centrosema Pubescens

G. A. WATSON

Centrosema pubescens and Mikania scandens were grown in pot culture in the presence and absence of nitrogenous fertilisers. In the absence of such fertilisers Centrosema pubescens produced good growth while Mikania scandens almost died out. A study of the nitrogen balance showed that Centrosema pubescens fixed appreciable quantities of nitrogen.

THE USE OF LEGUMINOUS CREEPERS as cover plants in rubber plantations and problems concerning nitrogen fixation by these plants under Malayan conditions have been discussed previously (WATSON 1957). Nitrogen fixation by these plants has already been demonstrated (HAMILTON & PILLAY 1941) but as an initial experiment, supplementary to a large programme of field experiments now being undertaken, a small scale pot experiment has been carried out to obtain some measure of the degree of nitrogen fixation effected by *Centrosema pubescens*, a leguminous creeper commonly used as a ground cover grown in association with rubber trees.

EXPERIMENTAL

The experiment was designed to compare the effects of the leguminous creeper *Centrosema pubescens* and the non-leguminous creeper *Mikania scandens* upon the soil nitrogen balance. Three surface cover treatments were used, namely *Centrosema pubescens*, *Mikania scandens* and no cover (bare soil). Four nitrogen treatments were applied: nil, and nitrogen equivalent to 4 cwt/acre of ammonium sulphate, applied as ammonium sulphate, sodium nitrate, or urea. The twelve treatments were carried out in duplicate.

Simple concrete cylindrical pots two feet tall and one foot in diameter were each filled with 100 lb of a gritty reddish yellow loam derived from granite, a Rengam series soil (OWEN 1951). Analytical details of this soil are:

	m. eq. per 100 gm	Organic carbon	Total nitrogen	pH
Exchangeable calcium	0.17			
Exchangeable magnesium	0.05	0.45%	0.052%	4.74
Exchangeable potassium	0.07			

A central drainage hole one inch in diameter in the bottom of the pot, protected from blockage by a pad of glass wool and a cover glass, was fitted with a rubber bung and glass tube and all leachates were collected in Winchester quart bottles.

The experiment was commenced in March 1956, when six *Centrosema pubescens* plants and six *Mikania scandens* plants per pot were established from inoculated seed, and from cuttings respectively. In the period from March to August three applications of fertiliser solutions were added to the pots. Nitrogen was added on each occasion in the quantity already mentioned, while phosphate, potassium and magnesium were added in solution to all pots each time in quantities equivalent to 2 cwt/acre of super-

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phosphate, 1 cwt/acre of muriate of potash, and 1 cwt/acre of magnesium sulphate respectively. Over the period of the experiment the plants depended for water on natural rainfall, and applied tap water added in equal amounts to all pots. All leachates were collected and analysed for ammonium and nitrate nitrogen.

Figure 1. A general view of the experiment.

The *Mikania scandens* plants rapidly produced vigorous growth in most of the pots and three cuttings of material were obtained; the *Centrosema pubescens* plants on the other hand developed more slowly and provided only sufficient material for two cuttings. All cut material was bulked at the end of the experiment, weighed and chemically analysed. At the same time all root, root nodule and leaf litter material was collected and separately weighed and analysed. Chemical analysis of the soil was carried out both before and after the experiment. Only the more pertinent of the analytical results are detailed in this paper.

RESULTS

The plants of both *Mikania scandens* and *Centrosema pubescens* grew well in the treatments receiving applications of nitrogenous fertilisers but only the *Centrosema pubescens* grew well in the absence of added nitrogen. *Mikania scandens* practically died out in this latter treatment and the marked difference in growth between the two plants is shown in *Figure* 2.

Soil analysis — Analysis showed that no differences existed between the treatments with regard to total soil nitrogen content either at the initiation or at the end of the experiment. At the end of the experiment the bare soil contained higher levels of nitrate nitrogen than did the soil under either cover plant, although the reverse was true for ammonium nitrogen (Table 1). This is interesting as a greater volume of leachate was obtained from the bare soil pots than from the others, and the difference may be accounted for either by a higher rate of nitrification of the ammonium nitrogen in the bare soil pots or by the uptake of nitrate by the plant roots in the other pots.

Another result of interest was that at the end of the experiment lower levels of exchangeable calcium and magnesium and of available phosphate were found in the soil of those pots which had received applications of ammonium sulphate fertiliser than of those which had received either sodium nitrate or urea fertiliser, irrespective of the cover plant treatment. In the case of magnesium the 0 to 6 inches layer of soil in pots receiving ammonium sulphate fertiliser contained lower levels than did the same zone of soil in pots receiving the other two fertilisers but the reverse was true for the soil below 6 inches, indicating that magnesium was being leached downwards in the ammonium sulphate treatments. In the case of calcium both the surface and lower layers of soil treated with ammonium sulphate contained lower levels of calcium than did those receiving the other fertilisers.

Plant analysis — Statistical analysis showed *Mikania scandens* to have significantly higher contents of calcium, magnesium, potassium, phosphorus and manganese than *Centrosema pubescens* (Table 2). This is possibly the first instance of a comparison of these two cover plants under experimental conditions and the results may eventually contribute towards an explanation of the plants' respective effects on growth of the rubber tree, now under investigation in field experiments. Both cover plants contained significantly higher levels of calcium in the absence than in the presence of applied nitrogen.

Leachate volume and nitrogen content — The volumes of leachate collected from each pot were totalled and found to reflect closely the treatment effects (Table 3). A significantly greater total volume of leachate was collected from the bare soil pots than from those carrying cover plants. In the *Centrosema pubescens* treatments no significant variation in leachate volumes was found, but in the case of those pots carrying *Mikania scandens* the 'no-nitrogen' pair of pots gave significantly greater volumes of leachates than did those receiving nitrogen fertiliser. This was a reflection of the extremely poor growth of the *Mikania scandens* in the absence of applied nitrogen.

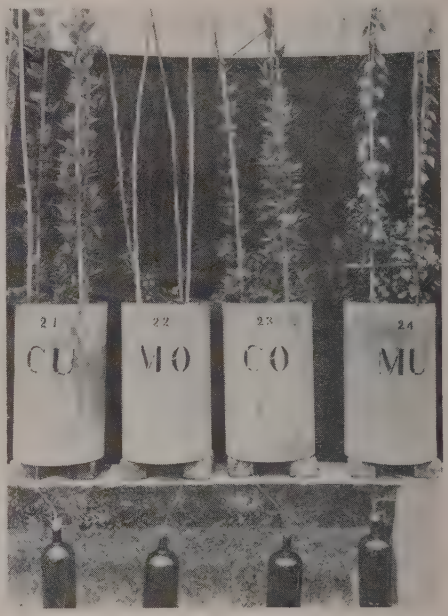

Figure 2. A comparison of Centrosema pubescens and Mikania scandens growing in the presence and absence of nitrogenous fertiliser.
CU = C. pubescens with applied urea
MU = M. scandens with applied urea
CO = C. pubescens with no fertiliser
MO = M. scandens with no fertiliser

Analysis of all leachates showed that the total amount of nitrogen leached from the bare soil pots was significantly greater than that leached from either of the cover-plant pots (Table 4). The amount of nitrogen leached from the pots carrying *Centrosema pubescens* was significantly greater than from those carrying *Mikania scandens* although a slightly greater volume of leachate was actually obtained from the latter treatment. A significantly greater quantity of nitrogen was leached from the pots receiving sodium nitrate fertilisers than from pots receiving either ammonium sulphate or urea, probably as a result of retention by the soil of ammonium ions in the latter two cases.

Nitrogen balance — To study the nitrogen balance in the different treatments the total nitrogen found in all plant materials and all leachates was calculated. These quantities showed important effects (Tables 4 and 5).

Summation of the total amount of nitrogen found in all leaf and stem material, all root and root nodule material together with any leaf litter for each treatment, showed that *Centrosema pubescens* plants contained a greater total amount of nitrogen than did *Mikania scandens* and that for both the mean total nitrogen found in plants receiving no nitrogen was significantly lower than the total in those plants which received nitrogenous fertilisers. For the *Centrosema pubescens* this effect was largely due to a significant increase in total nitrogen caused by the application of sodium nitrate, increases caused by the other two fertilisers only occurring to a non-significant extent.

The sum total of nitrogen found in all plant materials and leachates combined was significantly greater for the *Centrosema pubescens* pots than for the *Mikania scandens* pots. Subtraction of the amount of nitrogen leached from the bare soil pots, from the total amounts of nitrogen found in the plant materials and leachates of the cover plant pots (Table 5) shows that the *Centrosema pubescens* treatments contained a significant excess ($P<0.05$) of nitrogen over that leached from the bare soil pots, but that the *Mikania scandens* treatments contained a mean level of nitrogen slightly less than that value. In the absence of applied nitrogenous fertilisers the excess of nitrogen found in the *Centrosema pubescens* treatments amounted to a mean of 1723 mg/pot, while in the presence of applied nitrogenous fertilisers the mean excess amounted to 1138 mg/pot.

TABLE 1. NITROGEN IN SOIL AT END OF EXPERIMENT

Results in p.p.m calculated on oven dried soil, top 0 to 6 inches.

Form of nitrogen	Treatment	O	N	A	U	Means
			±0.2953			
Ammonium N	*C. pubescens*	3.745	3.925	3.315	2.565	3.388
	M. scandens	2.690	3.235	3.235	3.210	3.092
	Bare soil	2.435	2.080	2.790	2.290	2.399
	Means	2.957	3.080	3.113	2.688	
			±0.1705			±0.1475
			Min. 5% sig. diff. 0.531			Min. 5% sig. diff. 0.459
			±0.228			
Nitrate N	*C. pubescens*	1.390	1.790	1.325	1.680	1.546
	M. scandens	1.535	1.450	1.405	1.580	1.492
	Bare soil	1.790	1.960	1.535	2.665	1.988
	Means	1.572	1.733	1.422	1.975	
			±0.132			±0.114
			Min. 5% sig. diff. 0.411			Min. 5% sig. diff. 0.355

O = no nitrogen
N = nitrogen applied as sodium nitrate
A = nitrogen applied as ammonium sulphate
U = nitrogen applied as urea

DISCUSSION

Although the experiment allowed for a growing period of only some five months, several points with application to field conditions became apparent. The leaching downwards of calcium, magnesium and phosphate caused by the application of ammonium sulphate is of interest in view of the extent to which this fertiliser is used in rubber cultivation, although it must be realised that the experimental applications totalled the equivalent of 12 cwt/acre of ammonium suphate, a much higher amount than is used in a comparable time under field conditions. The greater recovery in leachates of nitrogen applied as sodium nitrate, when compared with the amounts recovered from applied ammonium sulphate and urea, is to be expected in view of the retention by the soil of ammonium ions.

The most important result of the experiment was the demonstration of an excess of nitrogen in the *Centrosema pubescens* treatments when compared with the *Mikania scandens* and the bare soil treatments. Analyses for total soil nitrogen were not taken into account in determining the nitrogen balance of the pots. This was because the total nitrogen content of the soil was so much higher than that of the plant materials and leachates (about 20,000 mg compared with about 3,000 mg, involving high multiplication factors in calculating the results of chemical analysis) that it was felt that analytical errors might mask treatment effects. There is an uncertainty therefore as

TABLE 2. ANALYSIS OF BULKED LEAF AND STEM MATERIAL FROM ALL CUTTINGS

Results calculated on oven dried materials.

Element	Treatment	O	N	A	U	Means
			±0.014			
P	*C. pubescens*	0.105	0.120	0.155	0.115	0.124
	M. scandens	0.250	0.180	0.185	0.180	0.199
%	Means	0.178	0.150	0.170	0.148	
			±0.010			±0.007
			Min. 5% sig. diff. 0.033			Min. 5% sig. diff. 0.021
			±0.029			
Mg	*C. pubescens*	0.090	0.095	0.110	0.090	0.096
	M. scandens	0.220	0.175	0.235	0.220	0.212
%	Means	0.155	0.135	0.172	0.155	
			±0.021			±0.015
			Min. 5% sig. diff. 0.070			Min. 5% sig. diff. 0.050
			±0.114			
K	*C. pubescens*	2.085	1.845	2.025	1.915	1.968
	M. scandens	3.080	1.810	2.605	2.670	2.541
%	Means	2.582	1.828	2.315	2.292	
			±0.0805			±0.057
			Min. 5% sig. diff. 0.269			Min. 5% sig. diff. 0.191
			±0.058			
Ca	*C. pubescens*	0.435	0.235	0.225	0.340	0.309
	M. scandens	0.750	0.470	0.455	0.480	0.539
%	Means	0.592	0.352	0.340	0.410	
			±0.041			±0.029
			Min. 5% sig. diff. 0.137			Min. 5% sig. diff. 0.097
			±14.08			
Mn	*C. pubescens*	83.5	79.5	110.0	77.5	87.6
p.p.m.	*M. scandens*	238.5	161.5	190.5	171.0	190.4
	Means	161.0	120.5	150.3	124.3	
			±9.95			±7.04
			Min. 5% sig. diff. 33.3			Min. 5% sig. diff. 23.55

to whether the excess of nitrogen found in the *Centrosema pubescens* pots was due to a more efficient exploitation by this plant of the applied nitrogen and nitrogen reserves in the soil, or whether this nitrogen was the result of fixation by the root nodule bacteria living in symbiosis with the legume. When it is considered that a greater quantity of nitrogen was leached from the pots carrying *Centromesa pubescens* than from those carrying *Mikania scandens* it seems unlikely that the former plant was making more effective use of the soil nitrogen content, unless it is postulated that the mineralisation of the soil organic nitrogen content was either being stimulated by *Centromesa pubescens* or depressed by *Mikania scandens*. Some plant roots have

TABLE 3. VOLUME OF LEACHATE PER POT COLLECTED OVER EXPERIMENTAL PERIOD

Millilitres

Treatment	O	N	A	U	Means
		±3015			
C. pubescens	43,338.5	40,226.5	42,159.5	42,528.0	42,063.5
M. scandens	72,194.0	33,853,5	32,685.0	32,097.0	42,707.4
Bare soil	81,913.0	80,930.0	83,364.0	83,301.5	82,377.1
Means	65,815.2	51,670.0	52,736.2	52,642.5	
		±1231			±1066
		Min. 5% sig. diff. 3831			Min. 5% sig. diff. 3318

TABLE 4. TOTAL NITROGEN IN LEACHATES AND PLANT MATERIAL

Miligrams

Source	Treatment	O	N	A	U	Means
			±58.87			
Total nitrogen in leachates	*C. pubescens*	175.5	777.5	327.0	429.5	427.4
	M. scandens	156.0	476.5	130.5	153.0	229.0
	Bare soil	474.5	2136.5	1981.5	1713.0	1567.4
	Means	268.7	1130.2	813.0	767.2	
			±33.99			±29.43
			Min. 5% sig. diff. 105.80			Min. 5%sig. diff. 91.6
			±200.7			
Total nitrogen in plant material	*C. pubescens*	2021.5	2953.5	2274.0	2484.0	2433.3
	M. scandens	163.0	1353.0	1770.5	1587.5	1218.5
	Means	1092.3	2153.3	2022.3	2035.8	
			±141.9			±100.3
			Min. 5% sig. diff. 474.6			Min. 5% sig. diff. 335.5

TABLE 5. NITROGEN EXCESS IN *CENTROSEMA PUBESCENS* TREATMENTS AS COMPARED WITH BARE SOIL TREATMENTS

Milligrams

Soil treatment	*Centrosema pubescens*				*Mikania scandens*				Bare soil			
	O	N	A	U	O	N	A	U	O	N	A	U
Mean total nitrogen/pot in plant material and/or leachates (x)	2197	3731	2601	2914	459	1830	1901	1741	475	2137	1982	1713
Total (x) minus nitrogen in leachates from bare soil treatment	1722	1594	619	1201	–6	–307	–81	28				

been reported as being able to affect the mineralisation of soil nitrogen (THERON 1951) but it would seem unlikely that such an effect could operate so consistently as the one under discussion in all treatments, both those receiving no nitrogen and those receiving nitrogen equivalent to 12 cwt/acre of ammonium sulphate, in the form of either ammonium sulphate, sodium nitrate or urea. Furthermore the result of analysis for ammonium and nitrate nitrogen in the soil give no support for the suggestion.

It seems highly probable therefore that the excess of nitrogen found in the *Centrosema pubescens* pots when compared with the other two treatments was the result of nitrogen fixation by the root nodule bacteria of the leguminous creeper. Examination had shown that the roots of these plants were well nodulated and that the nodules were particularly abundant around the air channels formed by stakes used to support the plants.

The mean excess of nitrogen was equivalent to 210 lb/acre of nitrogen in the absence of applied nitrogenous fertilisers and to 136 lb/acre of nitrogen in the presence of such fertilisers. In view of the marked difference in growth between the non-legume *Mikania scandens* and the leguminous creeper in the absence of applied nitrogenous fertiliser, and the fact that the excess nitrogen was produced in a period of only five months growth from the time of planting, the results are put forward as evidence that *Centrosema pubescens* can fix appreciable quantities of nitrogen.

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Soils Division
Rubber Research Institute of Malaya
Kuala Lumpur

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